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INTERNATIONAL SYMPOSIUM ON HODGKIN'S DISEASE

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INTRODUCTION

This International Symposium on Hodgkin's Disease is the fifth in a series of such symposia. The first one was organized by Professor Maurice Tubiana in Paris in February 1965, the second by the American Cancer Society, Inc., at Rye, New York, later in 1965, and the third by Professor D. W. Smithers at the Royal Marsden Hospital in London in 1968, and the most recent one, primarily devoted to clinical staging, was held at Ann Arbor, Michigan, in April 1971. It seemed appropriate to convene this Symposium at Stanford University this year because the first randomized clinical trial in the treatment of Hodgkin's disease was initiated just 10 years ago here at Stanford University in the Spring of 1962.

Our Symposium is supported by a grant from the International Union Against Cancer, which has helped to bring some of our distinguished participants from abroad, and by a grant from the National Cancer Institute, which has helped to bring some of the participants from this country. We are very grateful to both agencies for their very generous support.

The program clearly reflects a broadening of our perspectives about Hodgkin's disease. Although there are still many unsolved clinical problems in the areas of diagnostic evaluation, treatment, and prognosis, which were discussed in the latter part of the Symposium, the first three sessions were devoted to some new elements that have entered into our thinking. Unfolding work in the fields of geographic pathology and epidemiology, virology, immunology, and cytology has provided intriguing preliminary insights into what collectively might be called the biology of Hodgkin's disease. Thus the program reflects both a look at what has happened clinically in the past 10 years and the opening of a new and very exciting chapter in Hodgkin's disease research of a more fundamental character. It is highly appropriate that both clinicians and basic scientists were gathered together at this meeting because it is clear that they will have to work together to achieve the goals set by these new areas of investigation.

We arranged for the entire proceedings to be tape-recorded and have had the tapes transcribed, except for some segments of general discussion which proved inaudible and could not be transcribed. I have used these transcripts to prepare abbreviated summaries which paraphrase the discussion. It will be important, in reading these remarks, to remember that they are not a verbatim record of what was said. I hope, however, that the sense of each comment will be accurately reflected.

Henry S. Kaplan
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Stanford, California 94305

SESSION 1

EPIDEMIOLOGY AND ETIOLOGY

Part 1. Geographic Pathology of Hodgkin's Disease

Chairman: G. T. O'Conor

INTRODUCTION

In recent years there has been a dramatic surge of interest in the study of all aspects of Hodgkin's disease. Improved management has both contributed to and resulted from this interest. Now, increasing attention is being paid to the epidemiology and the etiology of this somewhat unusual form of cancer. In the first part of this Symposium, and at the Workshop on Epidemiology and Etiology later in this Symposium, we want to concentrate on the currently available data relating to geographic pathology, to discuss the significance of recent serologic and other immunologic observations, and to consider the possible etiologic role of certain viral agents. It is our hope and objective that the interrelationship of these 3 aspects will suggest new and testable hypotheses relating to etiology.

The first of these subjects is epidemiology and, more specifically, geographic pathology. Greatest attention has probably been given to the hypothesis proposed by B. MacMahon and P. Cole, who believe that geographic variations in age-specific incidence or mortality and differences in sex ratios are evidence that Hodgkin's disease is not a single entity, but rather 2 or more etiologically distinct processes. This is a very provocative and controversial hypothesis, which has stimulated tremendous interest in the epidemiology of this disease.

The so-called Rye modification of the Lukes-Butler classification has received fairly wide acceptance abroad as well as in the United States. The relationship of this classification to survival and its importance to prognosis have been very well established. Preliminary studies now indicate that histologic subtyping may also have epidemiologic significance, and, when correlated with geographic variations in age-specific incidence, may point to etiologic factors in the environment. At this time, we want to consider reports from various parts of the world in relationship to the histologic type, the relative frequency, and the age distribution curve of Hodgkin's disease to see whether this preliminary hypothesis holds up. It is, however, important that regional comparisons be made on a national basis in addition to the international comparisons. I would like to open this first session by presenting our review of the Connecticut State Registry material.

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Bethesda, Maryland 20014

Hodgkin's Disease in Connecticut: Histology and Age Distribution[1]

G. T. O'Conor,[2] P. Correa,[3] B. Christine,[4,5] L. Axtell,[3] *and* M. Myers,[3]
National Cancer Institute,[6] Bethesda, Maryland 20014

SUMMARY—The morphologic expression of Hodgkin's disease in the northeastern United States was characterized by review and histologic subtyping of 389 cases from the Connecticut State Tumor Registry according to the Rye classification. The sample represented 23% of cases recorded in Connecticut between 1935 and 1968. The age-specific relative frequency and the incidence rate for each histologic subtype were estimated. The patterns established from this survey in a population-based registry should serve as a basis for national and international comparisons. Geographic variations in histologic patterns related to age may provide additional evidence for the effect of environmental and host factors in etiology and suggest new testable hypotheses.—Natl Cancer Inst Monogr 36: 3–8, 1973.

THERE has been in recent years a rather surprising surge of interest in all aspects of Hodgkin's disease. Improved management has both contributed to and resulted from this interest, and increasing attention is being paid to the epidemiology and etiology of this relatively uncommon form of cancer.

Greatest attention has perhaps been given to the hypothesis of MacMahon, Cole and Aisenberg (*1–3*) that geographic variations in age-specific incidence of mortality and differences in sex ratios are evidence that Hodgkin's disease is not a single entity but rather 2 or more etiologically distinct processes. From an analysis of the available international incidence data and correlations with the Rye histologic subclassification of Hodgkin's disease in selected population groups, we are led to a different interpretation (*4*). Three epidemiologic patterns of Hodgkin's disease are described, but they appear to be a reflection of host response to environmental factors rather than an indication of separate and etiologically distinct diseases.

Pattern I is found in underdeveloped or developing countries and is associated with suboptimal socioeconomic conditions. Incidence rates are relatively high in children, and histologic subtypes associated with a poor prognosis are predominant. Pattern III is prominent in economically developed countries with high living standards. Although rare in children, there is a rapidly increasing incidence in young adults, and the histologic subtypes associated with a favorable prognosis are found in greatest frequency. Pattern II is intermediate in terms of the age-incidence curve and distribution of histologic subtypes. This pattern has been identified in the rural areas of western countries and may correspond to an intermediate stage in socioeconomic development and living standards.

The so-called Rye modification of the Lukes-Butler classification of Hodgkin's disease has received fairly wide acceptance abroad as well as in this country (*5, 6*). Recent studies have helped to establish the relationship of this classification to survival (*7–12*). As mentioned above, preliminary studies indicate that patterns of histologic type may also have epidemiologic significance and, when correlated with geographic variations in age-specific incidence, may point to etiologic factors in the environment.

---

[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Laboratory of Pathology.

[3] Biometry Branch.

[4] Connecticut Cancer Registry, Connecticut State Health Department, Hartford, Conn. 06103.

[5] We gratefully acknowledge the cooperation of the pathologists in the State of Connecticut who allowed us to review their slides and provided gracious assistance.

[6] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

It is important, therefore, that regional comparisons within countries be made in addition to international comparative studies of histologic distribution. All of the reported reviews of Hodgkin's disease which include histologic subtyping in this country and most from abroad utilized material from one hospital, and the emphasis has been on correlation with survival rather than on age-specific relative frequency. The purpose of this study was to establish, from a population-based registry, the age-specific relative frequencies and incidence rates for the histologic subtypes of Hodgkin's disease. We anticipate that it will serve as a standard for subsequent regional and international comparisons.

CASE MATERIAL AND METHODS

Connecticut has had a statewide cancer registry since 1935 that provides population-based data representative of the northeastern United States. The sample of cases for histologic review was selected so that all age groups were adequately represented. There were sufficient cases initially diagnosed between 1965 and 1968 to assess histologic type distribution for patients 20 through 69 years of age. For patients <20 and >70 years old, it was necessary to extend the sample to include all patients diagnosed from 1950 through 1968 (slide material was not consistently available for patients diagnosed before 1950).

The case material available, requested, and subsequently retrieved for review is listed in table 1 according to the specific age groups used for calculations of relative frequency. Between 1935 and 1968 there were 1,973 cases of Hodgkin's disease recorded in the Connecticut Tumor Registry. Appropriate material for histologic review from 550 cases was requested according to the schedule described above. For 95 of these cases, the material was either not found or was inadequate for study. We, therefore, ultimately reviewed 455 cases or a 23% sample of the total recorded cases in Connecticut. It should be particularly noted that the reviewed sample included over 50% of all patients <20 years old and nearly 50% of all patients ≧70 years old. It is interesting and of some significance that 66 or 15% of the cases reviewed were interpreted by us as not Hodgkin's disease and were therefore excluded from further analysis. The number and percentage of such disagreements are shown in table 2 according to age groups. Of the 66 non-Hodgkin's disease cases, 55 were patients ≧50 years of age. The explanation for this relatively high percentage of disagreement is largely a result of our more restrictive criteria for the diagnosis of Hodgkin's disease with lymphocyte depletion or Hodgkin's sarcoma. A high proportion of the misclassified cases recorded as Hodgkin's sarcoma were interpreted by us as reticulum cell sarcoma.

The histologic sections from all cases were

Table 1.—*Hodgkin's disease in Connecticut: Case material*

Age group (yr)	Cases recorded (1935–68)	Sample		% of total cases
		Requested	Retrieved	
0–14	91	1950–68— 58	52	55
15–19	132	1950–68— 93	73	55
20–34	535	1965–68— 90	82	15
35–49	408	1965–68— 61	53	13
50–69	563	1965–68—102	81	14
70+	239	1950–68—146	113	47
Unknown	2		1	50
	1,973	550	455	23

Table 2.—*Hodgkin's disease in Connecticut: Disagreement with recorded diagnosis*

Age group (yr)	Total cases	Not Hodgkin's disease	%
0–19	125	2	1.6
20–34	82	4	4.9
35–49	53	7	13.2
50–69	81	23	28.2
70+	113	30	26.6
Unknown	1	—	
	455	66	14.5

independently reviewed by 2 of us (G.T.O. and P.C.) and subclassified according to the Rye modification of the Lukes-Butler system as nodular sclerosis (NS), lymphocyte predominant (LP), mixed cellularity (MC), or lymphocyte depleted (LD). In most instances, the subclassification was made on biopsy material obtained before treatment. In 22 instances, the diagnosis of Hodgkin's disease was confirmed, but subclassification could not be made with confidence. These were classified as Hodgkin's disease, not otherwise specified (NOS). In many of these cases, the histologic appearance of the only material available had been altered by previous therapy. When the 2 reviewers disagreed on classification, the slides were restudied and a consensus was reached.

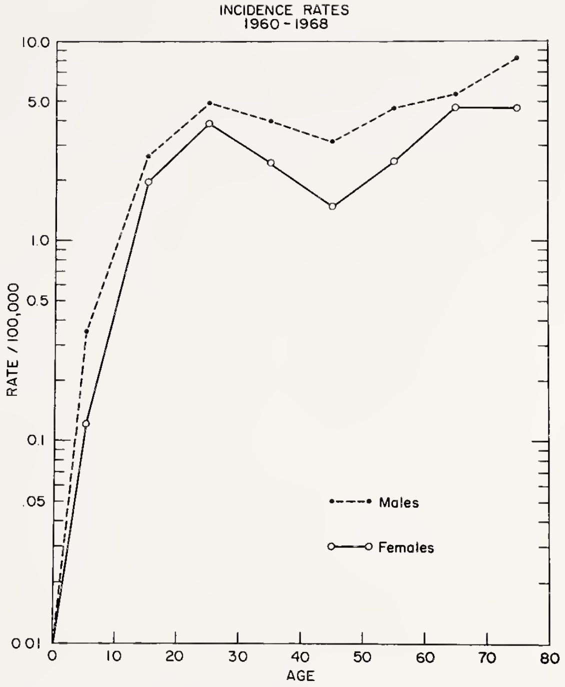
RESULTS

The sex ratio of the reviewed patient sample is shown in table 3 according to the specified age groups. There was slight to moderate male predominance in all age groups except adolescents (15–19 years old) and young adults (20–34 years old). These small differences were reflected in the age-specific incidence rates for males and females (text-fig. 1). The curves had a similar shape, with slight male predominance throughout life but with an exaggeration of this difference in the fifth decade.

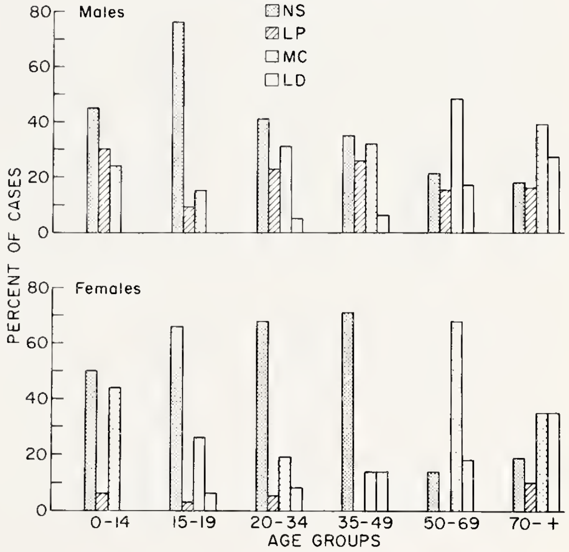
The number and percentage of cases reviewed are tabulated according to histologic subtype and by specified age group in table 4. Text-figure 2 presents the age-specific relative frequency of the histologic subtypes for each sex. It depicts most accurately the distinctive and characteristic age-distribution patterns for the different subtypes.

TABLE 3.—*Hodgkin's disease in Connecticut: Age and sex distribution*

Age group (yr)	Number of cases	Male/female ratio
0–14	51	1.8
15–19	72	0.9
20–34	78	1.1
35–49	46	2.2
50–69	58	1.3
70+	83	1.4
Unknown	1	–
	389	



TEXT-FIGURE 1.—Age-specific incidence rates of Hodgkin's disease for males and females.



TEXT-FIGURE 2.—Age-specific relative frequency of Hodgkin's disease by histologic subtype for males and females.

To further emphasize the presumed influence of age-related host and environmental factors,

Table 4.—*Hodgkin's disease in Connecticut: Histologic subtype*

Age group (yr)	NS #*	NS %	LP #	LP %	MC #	MC %	LD #	LD %	NOS #	NOS %	Total #
0–14	24	47	11	22	16	31	—	0	—	0	51
15–19	48	67	4	6	14	19	2	3	4	5	72
20–34	41	53	11	14	19	24	5	6	2	3	78
35–49	21	46	8	17	12	26	4	9	1	2	46
50–69	9	15	4	7	29	50	9	16	7	12	58
70+	14	17	10	12	28	34	23	27	8	10	83
Unknown	1	100	—	—	—	—	—	—	—	—	1
	158		48		118		43		22		389

* # = number of cases.

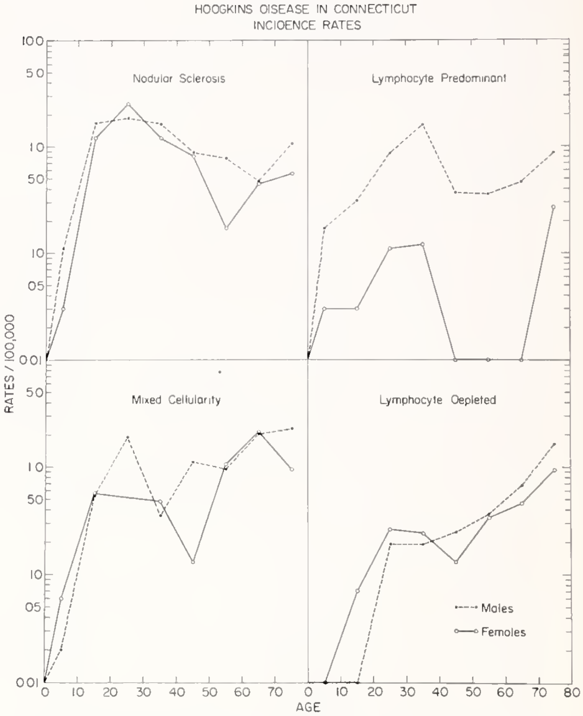
age-specific incidence rates were estimated for each histologic subtype. For this purpose calculations were made by applying the proportions in each histologic subtype found on review to each 10-year age-specific annual incidence rate covering the period from 1960 to 1968. These age-specific rate curves are shown in text-figure 3.

The illustrations of age-specific relative frequency and the incidence curves clearly characterize the pattern of Hodgkin's disease in the Connecticut population. NS was by far the most common form of Hodgkin's disease in adolescents, young adults, and adults into the fourth decade. There was a distinct peak in adolescence and early adulthood that accounted for the familiar first mode in the total incidence curve. Contrary to impressions drawn from hospital-based reviews, there was no apparent significant difference between male and female rates for NS. The greater dip in the female incidence curve for the 50- to 59-year-old group was probably specious. However, when expressed as relative frequency (text-fig. 2), the distribution of NS dropped at a later age in females than in males.

The LP form of Hodgkin's disease was relatively uncommon in all age groups and was chiefly a male disease. The peak incidence for both sexes was in the fourth decade with a marked dropoff thereafter until the very late years.

MC was the second most common form of Hodgkin's disease, and the incidence curves for male and females were bimodal and generally conformed to the shape of the total incidence curves (text-fig. 1).

Hodgkin's disease of the LD type afflicted older age groups and accounted for a significant portion of the second mode of the total incidence curve. Again, male and female rates differed only slightly.



Text-figure 3.—Age-specific incidence rates of Hodgkin's disease by histologic subtype for males and females.

DISCUSSION

Only a few reviews of Hodgkin's disease in this country have used the Rye classification. Lukes et al. (*8*) based their original classification on material collected at the Armed Forces Institute of Pathology. The patients were males, mainly of military age. Fuller et al. (*13*) reviewed at the M. D. Anderson Hospital and Tumor Institute in Texas 132 cases, but all of these were Stage I and Stage II. The series of Keller et al. (*9*) from the Stanford University School of Medicine in California included 176 cases but showed an age distribution in favor of young patients, which suggests some form of hospital selection. The largest group of cases studied before our survey was that of Berard et al. (*12*) from the Clinical Center at the National Institutes of Health (NIH). The 339 cases reviewed and subclassified in their report represented a selected hospital population group seen over a 16-year period.

The relative frequency of the 4 subtypes of Hodgkin's disease expressed as percent of total cases reviewed in the 3 hospital studies described above is compared with the Connecticut material in table 5. The figures in the bottom line were based only on cases diagnosed in 1965 through 1968 and, therefore, were devoid of the slight bias introduced by expanding our review to include additional cases from 1950 onward in the early and late age periods. In contrast to that from Connecticut, the series from the M. D. Anderson Hospital and Tumor Institute and from Stanford University had a high percentage of NS cases and a deficit of the LD type. In the former study, this could be related to the fact that the cases were all patients with Stage I and II disease while the age distribution of cases in the Stanford University series was markedly skewed. Table 6 shows the distribution of cases reviewed in 3 series, according to 4 age groups, compared with the age distribution of all cases recorded in the Connecticut Registry. The Stanford University series was overweighted with children and young adults, which explains the high frequency of NS. Only 10% of their cases were >50 years old, and this, in turn, probably accounts for the relative deficit of the LD type. On the other hand, the age distribution of the NIH Clinical Center cases also differed from that recorded in Connecticut. There was an excess of patients in the 15- to 34-year-old group and a deficit in the >50-year-old group. The relative frequency of histologic subtypes in the NIH study was nevertheless similar to that found in our review of the Connecticut material.

These comments are meant to emphasize the importance of age distribution when geographic comparisons of histologic subtypes are made and

TABLE 5.—*Subclassification of Hodgkin's disease*

Series	Total cases* classified	Histologic type (%)			
		NS	LP	MC	LD
M. D. Anderson Hospital and Tumor Institute	132	53	10	33	4
Stanford University	176	52	5	37	6
NIH Clinical Center	328	38	18	31	13
Connecticut (this review)	367	43	13	32	12
Connecticut slide (this review 1965–68)†	242	40	14	34	11

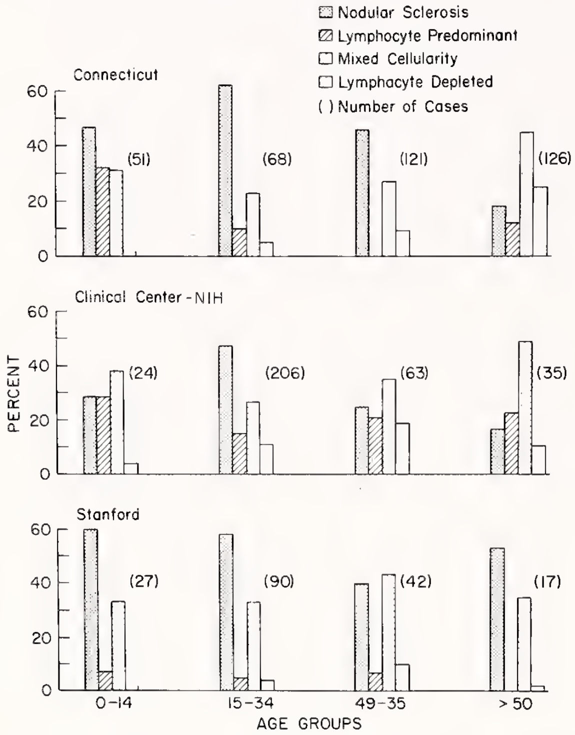
* Cases not subclassified (NOS) are omitted.
† There is no age bias in selection of the sample for years 1965–68.

TABLE 6.—*Hodgkin's disease: Comparative age distribution—percent of total cases*

Series	Number of cases	Percent in age group (yr)			
		0–14	15–34	35–49	≥50
Connecticut Registry (1935–1968)	1,973	5	34	21	40
Connecticut (this review)	389	13	39	12	36
NIH Clinical Center	339	7	62	19	12
Stanford University*	176	15	51	24	10

* Percent of cases in each age group is extrapolated from published curve to conform with age groupings in other series.

their epidemiologic significance is considered. Age-specific relative frequencies were calculated for the data presented in the reports from Stanford University and the NIH Clinical Center and graphically compared with our Connecticut material in text-figure 4. Although the effect of age bias in hospital selection was still apparent, the distortion was minimized and the similarities in pattern could be detected.



Text-figure 4.—Age-specific relative frequency of Hodgkin's disease by histologic subtype. Data from Stanford University, the NIH Clinical Center, and Connecticut are compared.

It is always difficult in epidemiologic studies of cancer, particularly when histologic parameters are included, to generate data which are population based. However, it is probable that age-specific relative frequency information can indicate the most important geographic differences in the morphologic expression of Hodgkin's disease. Such has been our experience in preliminary international studies. Before the present study, the histologic subtype pattern for Hodgkin's disease in the United States has been derived from individual hospital studies. The NIH Clinical Center study by Berard et al. (*12*) included the largest number of patients seen at any one institution. The results of our review of material from the population-based Connecticut registry are similar to those of the NIH study and, we believe, characterize the pattern of Hodgkin's disease in the northeastern part of the United States in terms of morphologic expression and age distribution. It is important that comparisons now be made on a regional basis within the United States, since mortality rates seem to vary from one region to another (*3*). We hope that correlation of different histologic patterns with epidemiologic parameters will suggest new and testable hypotheses relating to etiology.

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Hodgkin's Disease in Latin America [1]

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SUMMARY—Salient features of the epidemiology and geographic pathology of Hodgkin's disease in Latin America were described. We examined data on histologic subtypes from 10 countries (submitted to the Committee on Geographic Pathology of the International Union against Cancer by local pathologists), and incidence rates from 5 cancer registries. The data showed generally high rates in children, absence of the incidence peak described for young adults in Europe and the United States, predominance of histologic subtypes associated with poor prognosis, and a remarkably low frequency of the nodular sclerosis subtype of the disease. We correlated these with other parameters, such as per-capita income and death rates for tuberculosis.—Natl Cancer Inst Monogr 36: 9–14, 1973.

HODGKIN'S disease is common in Latin America, and several reviews of its clinical and pathologic aspects have been published (*1–5*). It is usually described as a rapidly progressive disease which affects the superficial lymph nodes and is often associated with tuberculosis. It frequently occurs in children (*4, 5*). This paper reviews age distribution patterns and histologic subclassifications based on recent information from several cancer registries and pathology departments throughout Latin America.

MATERIALS AND METHODS

This study used 2 kinds of data: 1) relative frequencies of histologic subtypes and 2) incidence rates.

Relative frequency of histologic subtypes.—These data were kindly supplied to the Committee on Geographic Pathology of the International Union Against Cancer by a number of collaborating pathologists. The histologic subtypes were specified for each sex and 5-year age groups. The Committee collected data on all lymphomas, analyzed in (*6*). Criteria for the histologic subclassification were discussed and illustrated in a special conference attended by most of the participating pathologists. The conference tested the reproducibility of the classification by several observers, with satisfactory results (*6*). The Rye classification was used, with cases classified as nodular sclerosis (NS), lymphocyte predominant (LP), mixed cellularity (MC), or lymphocyte depleted (LD). The data were submitted by the following collaborators:

1) Dr. Francisco Platero, Hospital Rosales, El Salvador. A charity hospital excluding workers with State health insurance and wealthy patients.
2) Dr. Jorge Salas, Hospital San Juan de Dios, San Jose, Costa Rica. A charity hospital receiving approximately 80% of the pathology material of Costa Rica.
3) Dr. Francisco Martin and Dr. Armando Santamaria, Instituto de Cancerologia, Bogota, Colombia. A charity hospital.
4) Dr. Victor Bedoya, Hospital Universitario, Medellin, Colombia. A charity hospital.
5) Dr. Carlos Cuello, Hospital Universitario, Cali, Colombia. A charity hospital.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

6) Dr. Oscar Misad, Instituto de Enfermedades Neoplasicas, Lima, Peru. A charity hospital.
7) Dr. Pedro Albujar, Hospital Belen and Hospital Regional, Trujillo, Peru. Charity hospitals providing about 90% of the pathology services for that area of Peru.
8) Dr. Adonis de Carvalho, Hospital Universitario and Hospital de Cancer, Recife, Brazil. Charity hospitals.
9) Dr. J. Carlos Machado, Hospital de Cancer, São Paulo, Brazil. Both charity hospital and hospital for private patients.
10) Dr. Alfonso Martinez, Hospital Universitario, Concepcion, Chile.
11) Dr. Santiago Besuschio, Academia of Medicina, Buenos Aires, Argentina. Material is from the following large hospitals of Buenos Aires: Institute of Hematologic Investigations; Municipal Institute of Hematology; Oncological Institute. Cases from the General Registry of Pathology are included also. The Registry provides pathology services to small hospitals.

Incidence rates.—Age-specific incidence rates were kindly supplied by Dr. José Galvez Brandon (Lima, Peru), Dr. Bertoldo Kruse (Recife, Brazil), and Dr. Isidro Martínez (San Juan, Puerto Rico).

RESULTS

The results are summarized in table 1, with data from Connecticut included for comparison. We have adjusted for differences in population composition to avoid distortion in comparisons of the relative frequency distribution of cases by age. We did this by relating the actual proportion of cases observed in each age group to the hypothetical proportion expected if the disease were homogeneously distributed over all age groups (O/E ratio). Connecticut's 0.29 ratio would indicate that the actual frequency of the disease in children is approximately a third of the expected frequency if the disease were equally distributed in all age groups. Higher ratios would indicate, therefore, a relative increase in the proportion of cases not accounted for by differences in the population structure. The first column in table 1 ranks the data by O/E ratios in 0- to 19-year-old males. The O/E ratios for females and other age groups did not show meaningful comparisons. The next 3 columns refer to sex ratios in the 3 age groups, and the last column shows the ratio of the more benign to the more malignant subtypes in the sample reviewed histologically. We can divide the results into 3 main groups. The first includes data from Peru, Colombia, and El Salvador. These countries have the highest ratios in children. The sex ratios (M/F) are also very high in children and decline as age advances (with 1 exception). The ratio of histologic subtypes indicates a predominance of the worst prognostic types (again with 1 exception).

TABLE 1.—*Hodgkin's disease in Latin America and Connecticut*

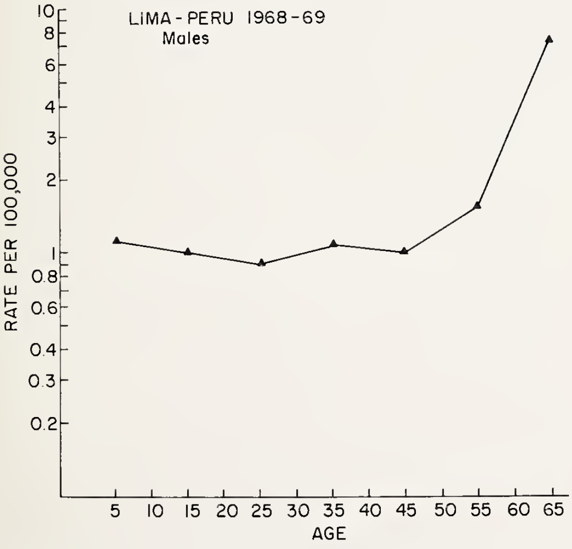
Location (yr)	O/E, males, 0–19 yr old	Sex ratio: males/females			
		0–19 yr old	20–49 yr old	≥50 yr old	Ratio: NS–LP/MC–LD
Lima, Peru (1952–70)	0.97	3.6	2.7	1.3	0.39
Bogota, Colombia (1955–70)	.89	3.0	3.4	1.4	.56
Cali, Colombia (1962–69)	.73	5.7	2.4	1.5	.38
El Salvador (1961–70)	.72	2.6	2.2	2.2	.29
Trujillo, Peru (1960–67)	.69	6.0	2.7	1.3	.25
Medellin, Colombia (1944–69)	.68	3.4	3.1	1.5	.82
Recife, Brazil (1961–70)	.64	2.3	2.1	1.4	1.40
São Paulo, Brazil (1961–70)	.65	3.7	2.3	1.9	0.59
San Jose, Costa Rica (1960–64)	.59	4.0	2.5	2.7	.46
Buenos Aires, Argentina (1957–70)	.49	1.7	1.5	2.3	.62
Concepcion, Chile (1960–69)	.39	3.0	4.0	2.2	
Connecticut (1950–68)	.29	1.1	1.5	1.5	1.4

Group 2 includes Brazil and Costa Rica, which are similar to group 1 but show less constant results, probably due to small sample size. The decline of sex ratios with age is less steep and the predominance of poor prognostic subtypes is more variable.

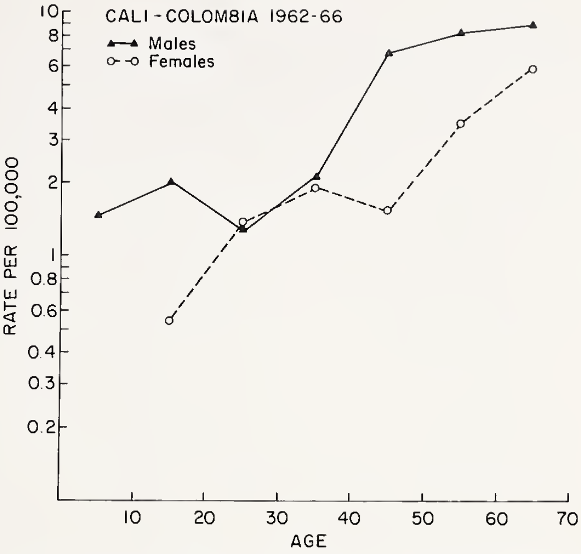
The last group, including Chile and Argentina, shows a pattern which approximates that of Connecticut. Ratios are relatively lower in children; sex ratios show no decline with age; there are fewer cases of the subtypes that have poor prognosis.

Table 2 shows the results of the histologic review of cases. The proportion of NS subtype is very low when compared with reports from the United States and Europe. This characteristic yields area rankings that generally agree with those shown in table 1. The small number of cases makes it difficult to draw inferences from the data for individual age groups. The slides from Trujillo, Peru, were reviewed by pathologists at the National Cancer Institute and represent all cases of Hodgkin's disease diagnosed during the study period.

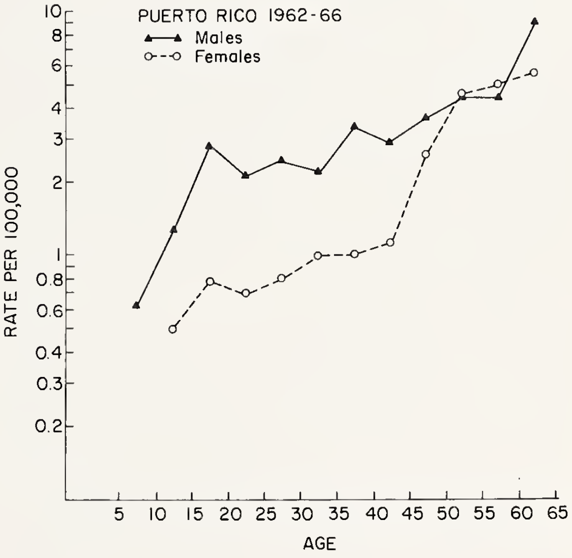
Text-figures 1–5 show the age-specific incidence rates from registries of Latin American populations. The number of female cases in Lima was so small that it is omitted.



Text-figure 1.—Age-specific incidence rates for males of greater Lima, Peru. 1968–69. Source: Cancer Registry of Lima, Dr. José Galvez Brandon.



Text-figure 2.—Age-specific incidence of Hodgkin's disease in Cali, Colombia. 1962–66. Source: Cancer Registry of Cali.

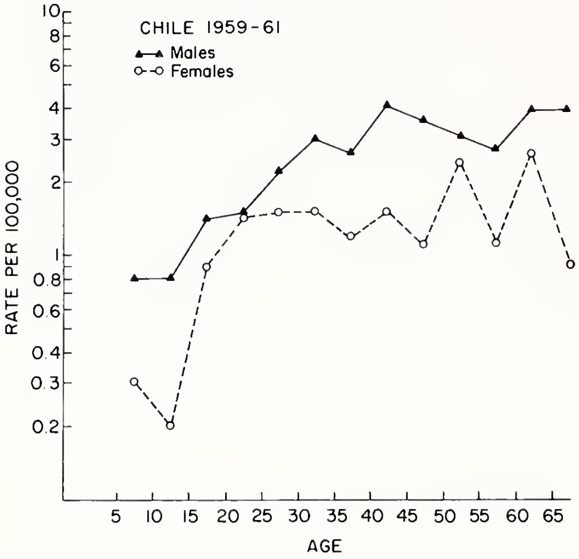


Text-figure 3.—Age-specific incidence of Hodgkin's disease in Puerto Rico. 1962–66. Source: Cancer Registry of Puerto Rico, Dr. Isidro Martínez.

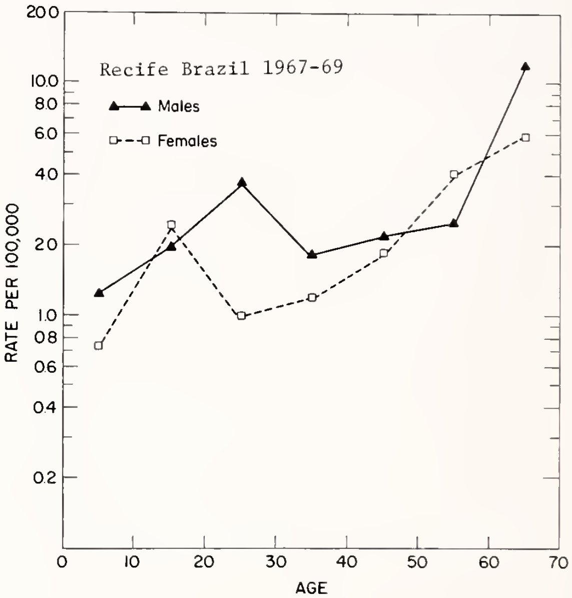
The incidence curves from Peru and Colombia show a high rate in children, without the incidence peak described for young adults in the United States and Europe. In Recife the incidence in children is also high, and it peaks in young-adult males and adolescent females. The

TABLE 2.—*Percent of subtypes of Hodgkin's disease*

Location	Males				Females				Number of cases	
	NS	LP	MC	LD	NS	LP	MC	LD	Males	Females
Lima, Peru	5	23	44	27	8	11	46	35	155	65
Trujillo, Peru	7	13	13	67	0	0	29	71	18	7
Bogota, Colombia	13	21	55	11	17	19	47	17	147	53
Cali, Colombia	13	14	49	24	14	23	54	9	78	22
Medellin, Colombia	17	29	36	18	12	25	36	26	280	98
El Salvador	13	9	67	10	27	5	52	15	112	47
San Jose, Costa Rica	14	17	54	14	17	25	33	25	35	12
Recife, Brazil	22	32	32	15	31	27	27	15	88	48
São Paulo, Brazil	18	19	52	10	41	2	52	4	87	44
Buenos Aires, Argentina	16	25	49	10	30	14	42	13	313	188
Connecticut, United States	39	20	31	10	48	4	33	14	210	157



TEXT-FIGURE 4.—Age-specific incidence of Hodgkin's disease in Chile. 1959–61. Source: Doll R, Payne P, Waterhouse JA, eds.: International Union Against Cancer: Cancer Incidence in Five Continents, vol 1. Berlin, Heidelberg, New York, Springer-Verlag, 1967.



TEXT-FIGURE 5.—Age-specific incidence of Hodgkin's disease in Recife, Brazil. 1967–69. Source: Cancer Registry of Pernambuco, Dr. Bertoldo Kruse.

rates in Chilean children are also moderately high, and the adult rates rise progressively until age 40. In Puerto Rico the rates in children are lower than those in the other Latin American communities but higher than in the United States or Europe; the incidence peak occurs in adolescence.

DISCUSSION

Our findings confirm previous reports of marked geographic differences in the epidemiology and pathology of Hodgkin's disease in several areas (*7–9*). The data from Peru, Colombia, and Central America seem to represent epidemiologic pattern I: a high incidence rate in children, an absence of the young-adult peak, and a predominance of the histologic subtypes associated with poor prognosis (7). None of the Latin American countries studied present the epidemiologic pattern III of the United States and Western Europe. Argentina and Chile approach this pattern with their small proportion of childhood cases, but neither country shows an incidence peak in young adults or a predominance of the NS subtype. Puerto Rico also presents intermediate pattern II. Earlier data from Puerto Rico showed a higher incidence in children. The data from Recife are unique in that they show high rates for children of both sexes and a peak in young-adult males. These results may reflect the small size of the sample or some other demographic characteristic.

Incidence and relative frequency of histologic subtypes correlate roughly with latitude. The tropical countries, such as Peru, Colombia, Costa Rica, and El Salvador, have higher rates in children and a marked predominance of the histologic subtypes associated with poor prognosis. The temperate countries of Argentina and Chile have much fewer cases in children. There seems to be a correlation, also, with such other variables as per-capita income (table 3) and the death rates from such chronic diseases as tuberculosis (table 4), though no precise parallels can be drawn. For per-capita income, the ranking order of table 3 follows that of tables 1 and 2, except for Brazil. For tuberculosis, the ranking order of table 4 also corresponds to tables 1 and 2. The only exception is Chile, probably due to the unusually high rate of tuberculosis in adult males. Tuberculosis rates for children are lower in Chile than in Peru and Colombia (*10*). Tuberculosis in Latin American children is practically always associated with malnutrition (*11*) and is also frequently seen in children with Hodgkin's disease (*2*). Therefore, it seems reasonable to speculate that pattern I may be associated with poverty, malnutrition, and tuberculosis.

We interpret our data as supporting our contention that the natural history of Hodgkin's disease is strongly influenced by economic conditions which influence nutritional state and the prevalence level of other chronic infectious diseases such as tuberculosis (7). This might indicate that the immune capacity of the host influences the development and clinical course of Hodgkin's disease.

TABLE 3.—*Per-capita income in some Latin American countries and the United States. (Gross national product per capita in U.S. dollars, data for 1963. From World Population Data Sheet, 1966)*

Country	Dollars
Brazil	196
Peru	262
Guatemala	284
Colombia	292
Costa Rica	385
Chile	483
Argentina	614
United States	3,083

TABLE 4.—*Annual age-adjusted death rates for tuberculosis per 100,000 population at ages 15–74; 1962–64. From Puffer and Griffith (8)*

Location	Rate
Lima, Peru	67.0
Santiago, Chile	54.8
Cali, Colombia	47.5
Guatemala City	36.4
Bogota, Colombia	25.6
São Paulo, Brazil	18.7
La Plata, Argentina	8.8
San Francisco, United States	7.0

This is only a preliminary investigation of Hodgkin's disease in Latin America, but it seems to indicate extensive clinical, epidemiologic, and etiologic studies may yield valuable scientific information.

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Hodgkin's Disease in Asia, Particularly in Japan [1,2]

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SUMMARY—The incidence and histologic subtypes of Hodgkin's disease in Asia, particularly in Japan, were reported from the standpoint of geographic pathology. A crude death rate obtained from vital statistics showed a higher occurrence of Hodgkin's disease in the southern area of Japan. This fact was also supported by the age-standardized rate of Hodgkin's disease in 2 registries. The annual autopsy report showed that Hodgkin's disease had a remarkably low incidence compared with other types of lymphoma. For a study of the distribution of histologic subtypes of Hodgkin's disease in Japan, the 166 cases collected for the present investigation were reviewed according to the Rye classification. Mixed cellularity was the most common form in males (42%); nodular sclerosis was most common in females (38.3%). Nodular sclerosis was infrequently observed, and mixed cellularity had the highest incidence. —Natl Cancer Inst Monogr 36: 15–22, 1973.

A DIFFERENCE in incidence of malignant lymphoma and leukemia between the United States and Japan has been observed. In general, there is a strikingly low incidence of lymphocytic leukemia, lymphosarcoma, and Hodgkin's disease and a preponderance of reticulum cell sarcoma in Japan. In contrast, lymphocytic lymphoma and Hodgkin's disease occur frequently in the United States. For an explanation of such variation, strict criteria should be applied in the differentiation of these diseases.

The present paper reports on epidemiologic and pathologic characteristics of Hodgkin's disease in Asia, particularly in Japan, with special reference to other types of malignant lymphomas.

PROCEDURES AND RESULTS

Japan

Japan consists of 4 major islands, and lies between 44–24° north latitude. The northern area is cold and has much snow in winter, but the southern area is warm throughout the year.

Crude Death Rate

The crude death rates obtained from vital statistics indicated a different frequency of Hodgkin's disease in different parts of Japan (*1*). The mean death rate from Hodgkin's disease was 0.64/100,000 in males and 0.36 in females. Although death certificates were not completely based on histologic diagnosis, rates were higher in both males and females in the southern area of Japan (text-fig. 1).

Consequently, 2 districts of this country, Tohoku, in the northern part of the main island, and Kyushu, a southern island, were chosen for a comparison of the ratio of Hodg-

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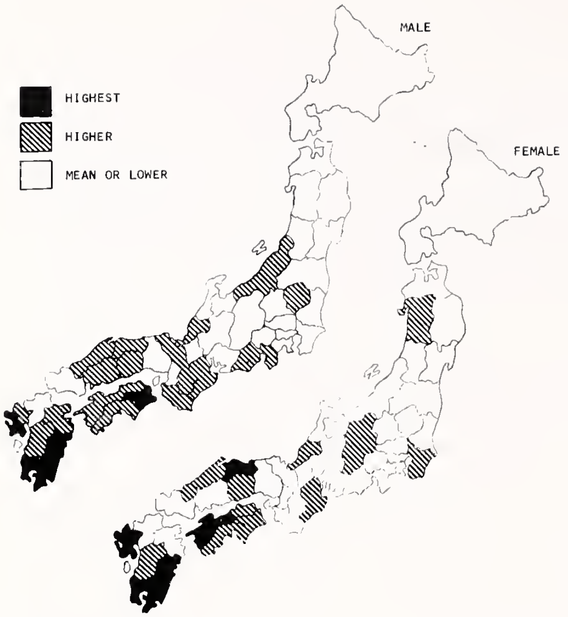
[3] Former Dernham Fellow, American Cancer Society, Inc., California Division.

[4] I thank Drs. A. Piankijagum, S. C. Sun, K. Kageyama, and R. J. Lukes for their assistance. I am grateful to the Departments of Pathology of Kyushu, Hiroshima, Jikei, Niigata, Akita, and Hirosaki Universities, Fukushima and Iwate Medical Colleges, Aichi Cancer Center, and Iwate Central Hospital.


MALE
FEMALE
HIGHEST
HIGHER
MEAN OR LOWER

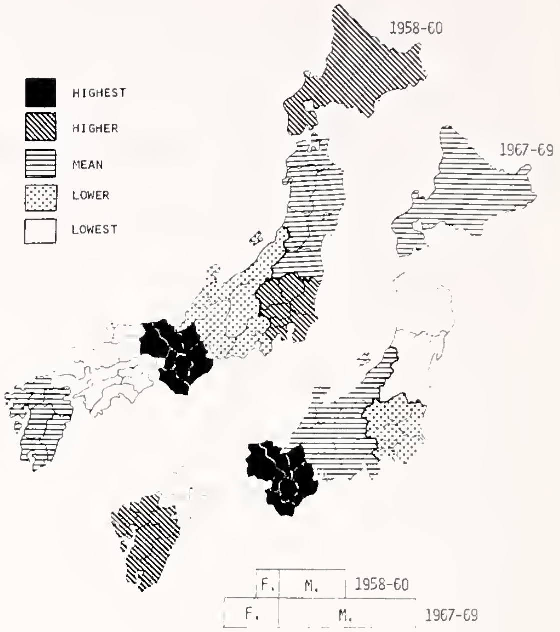
Text-figure 1.—Comparison of death rates for Hodgkin's disease in different parts of Japan from 1958 to 1967. Data are for males and females/100,000 population.

kin's disease to the total number of malignant neoplasms. The ratios are 0.84% in males and 0.72% in females in Kyushu and 0.46% and 0.28% in Tohoku. Leukemia and other types of lymphoma are also more frequently observed in Kyushu.

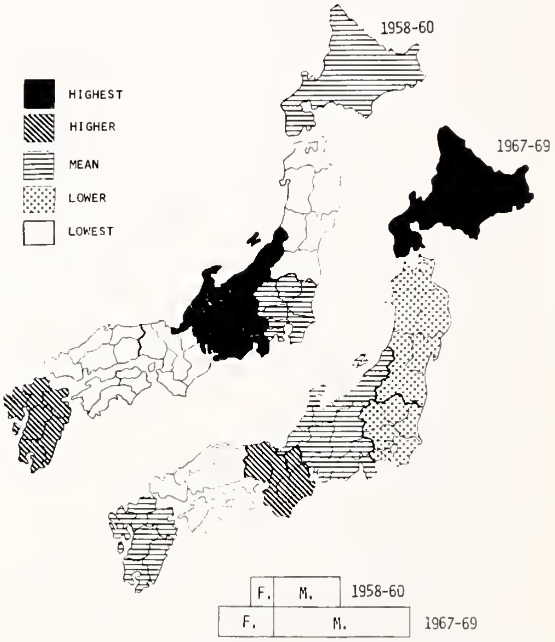
Autopsy Data

In Japan, autopsy data at medical schools, institutes, and major hospitals are registered at the Pathological Society and published annually (*2*). Maps based on the record during 2 periods, 1958–60 and 1967–69, represent the crude autopsy rate for Hodgkin's disease in different areas (text-figs. 2, 3). The slight difference in the occurrence of the disease, as shown in text-figures 1, 2, and 3, may be due partly to a discrepancy in autopsy rates of Hodgkin's disease at different institutions. The number of autopsies has increased in the past 3 years.

For each 3-year period, 3 major types of malignant lymphoma in the autopsy data report (*2*) were compared by the following formula: No. of deaths from each type of lymphoma ÷


1958-60
1967-69
HIGHEST
HIGHER
MEAN
LOWER
LOWEST
F.
M.
1958-60
F.
M.
1967-69

Text-figure 2.—Comparison of incidence of Hodgkin's disease in males from crude autopsy rates in different parts of Japan during the periods 1958–60 and 1967–69.


1958-60
1967-69
HIGHEST
HIGHER
MEAN
LOWER
LOWEST
F.
M.
1958-60
F.
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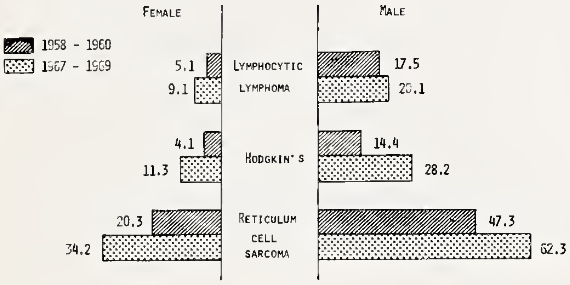
1967-69

Text-figure 3.—Comparison of incidence of Hodgkin's disease in females from crude autopsy rates in different parts of Japan during the periods 1958–60 and 1967–69.

total No. of autopsies × 10,000. In the 3 years 1958–60, reticulum cell sarcoma had the highest and Hodgkin's disease the lowest incidence in both sexes. In the 3 more recent years, the order of incidence from highest to lowest was reticulum cell sarcoma, Hodgkin's disease, and lymphocytic lymphoma (text-fig. 4).



Text-figure 4.—Comparison of incidence of 3 types of malignant lymphoma in males and females in Japan during the periods 1958–60 and 1967–69. (*See* text for formula used.)

Pathology

From 71,266 surgical specimens, 186 cases of malignant lymphoma were obtained from the files of the Department of Pathology, Tohoku University, for the 8 years from 1964 to 1971. The slides were routinely stained with hematoxylin and eosin, and occasionally with a special stain. They were then arranged according to the classification for histologic types proposed by Lukes (*3*) (table 1). Hodgkin's disease was observed infrequently (only 14.5%) in the files of surgical specimens.

We collected 166 cases of Hodgkin's disease from the surgical materials of more than 10 medical institutions in Japan. They are not closely correlated with the population base in each district.

The criterion for diagnosis was the presence of Reed-Sternberg giant cells. We used the Rye classification of histologic subtypes: lymphocytic predominance (LP), mixed cellularity (MC), lymphocytic depletion (LD), and nodular sclerosis (NS).

LP has a diffuse proliferation of lymphocytes mingled with mononuclear histiocytes and a small number of Reed-Sternberg giant cells (fig. 1).

MC, a usual evolution from LP, is characterized by an increased number of Reed-Sternberg giant cells and fibrosis with decreased numbers of lymphocytes (fig. 2).

In LD, the lymphocytes disappear, as observed in the reticular and diffuse fibrosis types originally proposed by Lukes et al. (*4*) (figs. 3A, B).

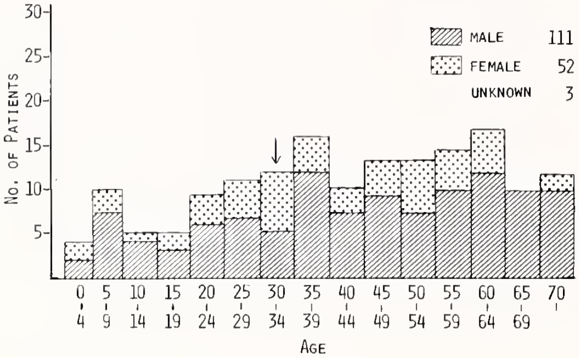
NS has a different appearance, and retains its morphologic features constant throughout its course (*5*). It has thin or thick collagen bundles and lacunar cells combined with Reed-Sternberg giant cells (fig. 4). Because of the high frequency of NS in the United States, we carefully researched this type of Hodgkin's disease in the Japanese material. We included not only advanced stages but also early stages containing thin collagen bundles, lacunar cells, and abundant lymphocytes.

Of the 166 patients examined, 113 were males

Table 1.—*Histologic types of malignant lymphona at Tohoku University between 1964 and 1971*

Histologic type	Number of nodular cases	Number of diffuse cases	Total cases (%)
Lymphocytic			
Well differentiated	0	9	4.9
Poorly differentiated	15	7	11.8
Stem cell or undifferentiated cell	0	29	15.6
Reticulum cell or histiocytic	25	74	53.2
Hodgkin's disease	27		14.5
Total No. of cases	186		
Total surgical specimens	71,266		

and 53 were females; the sex ratio was 2.19:1. Text-figure 5 shows the age distribution, with the exception of 3 patients whose ages were unknown. The cases are almost evenly distributed in all age groups. In the 30- to 34-year group, the number of females (*6*) is higher than that of males (*5*). NS was observed in 5 of the 7 females.



Text-figure 5.—Age distribution for Hodgkin's disease in Japan. In 3 of 166 cases, ages were unknown. *Arrow* points to 30- to 34-year group, in which the number of cases in females is higher than that in males.

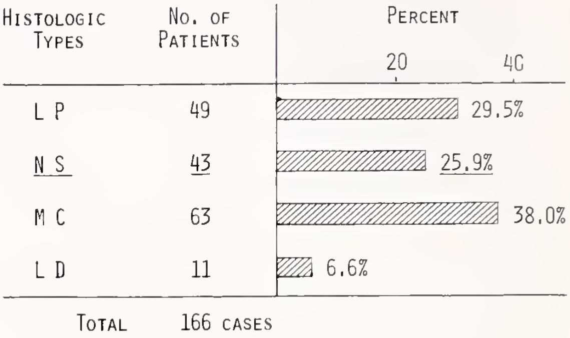
NS in females occurred at a younger median age than the other histologic subtypes (table 2). The frequency of subtypes differed according to sex: In males, MC was the most common (42.1%); in females, NS (38.3%) was the most common. However, there was no sexual difference in frequency of NS.

Table 2.—*Median age for occurrence of Hodgkin's disease in Japan*

Histologic type	Males (yr)	Females (yr)
LP	39	53
NS	36	32
MC	55	39
LD	50	44*

* Three cases only.

The distribution of histologic subtypes among the total cases of Hodgkin's disease is presented in text-figure 6. MC showed the highest occurrence, and LP was next. Distribution of histologic subtypes differed between the United States and Japan.



Text-figure 6.—Incidence of histologic subtypes of Hodgkin's disease.

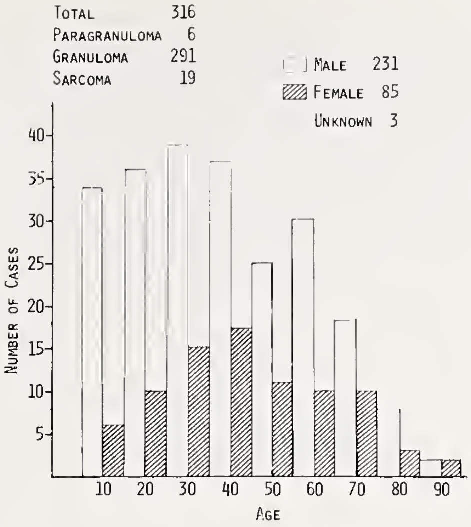
Asia Other Than Japan

It was impossible to gather much information about Hodgkin's disease from many countries outside Japan because of poor communication. In Thailand, Piankijagum (*7*) reported that Hodgkin's disease and lymphoma were found in 1,094 of 140,314 surgical specimens at the Department of Pathology, Siriraj Hospital, during the 15 years from 1957 to 1971. The frequency of Hodgkin's disease was 28.9% of all lymphomas, and ranked second after frequency of reticulum cell sarcoma, which was 43.2%. The cases were evenly distributed in all decades of life (text-fig. 7).

In Taiwan, Sun (*6*) reported that Hodgkin's disease had a low frequency among total surgical specimens at the Veterans General Hospital (0.033%) and the Tri-service Army Hospital (0.028%). These hospitals had different peaks of age distribution—at the former, the peak was observed in older ages than at the latter (text-fig. 8).

DISCUSSION

In Asian countries outside of Japan, the different incidence of Hodgkin's disease in relation to other malignant lymphomas may be due to diagnostic and cytologic criteria or to biologic differences in the population. In Thailand and Singapore, Hodgkin's disease occurred less frequently than reticulum cell sarcoma, but



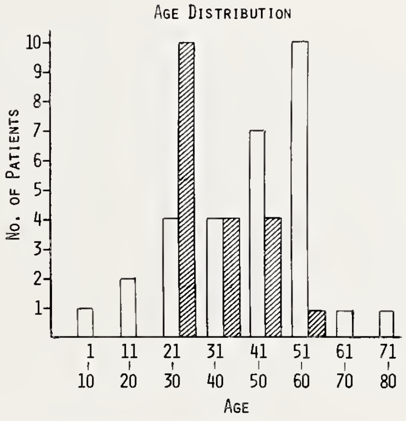
Surgical specimens 140,314 (1957-71)

Hodgkin's disease and lymphoma 1,394

Hodgkin's disease	28.9%
Reticulum cell sarcoma	43.2%
Lymphosarcoma	25.9%
Lymphoma(Unclassified)	5.1%

Courtesy of Dr. A. Piankijagum

TEXT-FIGURE 7.—Frequency of malignant lymphoma in Thailand between 1957 and 1971. The incidence in males was higher than that in females, with a ratio of 2.7:1.



Veterans General Hospital ☐

Surgical specimens 89,820(1962-71)

Hodgkin's disease 30(0.033%)

Sex ratio M : F :: 2 : 1

Tri-service Army Hospital ▨

Surgical specimens 681(1961-71)

Hodgkin's disease 19(0.028%)

Sex ratio M : F :: 17 : 2

Courtesy of Dr. Shih-Chien SUN

TEXT-FIGURE 8.—Incidence of Hodgkin's disease in Taiwan. Surgical specimens were from Veterans General Hospital (1962–71) and Tri-service Army Hospital (1961–71).

more frequently than in the present series in the Department of Pathology, Tohoku University School of Medicine.

In Japan, the incidence of both leukemia and malignant lymphoma has gradually increased during the past 2 decades (*8*). In the United States, death rates for leukemia, lymphoma, and Hodgkin's disease were almost 3 times higher in all the combined races than in Japan (*19*). Our study showed a remarkable difference in the frequency of each cytologic type of lymphoma, particularly reticulum cell or histiocytic type and Hodgkin's disease.

The different death rates for Hodgkin's disease in the northern and southern areas of Japan were obtained from vital statistics. This difference was also supported by data for age-standardized rates in 2 registries of Miyagi and

Okayama. In Miyagi prefecture in the north, the death rates for Hodgkin's disease were 0.5 in males and 0.4 in females (adjusted to the European population) or 0.4 and 0.3 (adjusted to the world population). However, in the Okayama prefecture in the south, the death rates were 1.4 or 1.0 in males and 0.8 or 0.6 in females (*10*).

A slight difference in the distribution of Hodgkin's disease was observed between the crude death rates and the annual autopsy report. Two possible reasons for this discrepancy are autopsy rates for Hodgkin's disease and inconsistency between clinical and anatomic diagnosis. A reverse occurrence for Hodgkin's disease and sarcoidosis has been reported—i.e., the sarcoidosis rate was higher in the northern area (*11*). However, whether a different climate may have some correlation to the incidence of Hodgkin's disease must be studied further.

The size of the Reed-Sternberg giant cell nucleolus is the most convincing criterion for diagnosis of Hodgkin's disease. The peak frequency of the nucleolar area was $15\mu^2$ (*12*). Three of the four histologic subtypes of the Rye modification are based on their cellular components, especially the numbers of lymphocytes and histiocytes. NS Hodgkin's disease, however, is characterized by its structural features, particularly the formation of collagen bundles.

Incidence of NS in Americans ranged from 40% (*4*) to 70% (*13*). Lukes et al. reported cases mainly in males of a relatively young age (*14*). NS was observed predominantly in females <35 years old in the United States. In our studies, this subtype occurred in 25.9% of the total cases, but the highest rate, 38.3%, was in females. Thus, the distribution of histologic subtypes in Japanese clearly differs from that in Americans. These variations in incidence and distribution may be due to genetic differences.

An increasing number of reports during the past 5 years correlate prognosis with histologic subtypes of the Rye modification of the classification originally proposed by Lukes et al. (*4*). Survival of cases with MC and LD was poor.

Unfortunately, most of the cases in the present study had poor clinical information. Therefore, we could not correlate histologic types with clinical staging and prognosis. Close cooperation between pathologists and clinicians is important in the study of Hodgkin's disease.

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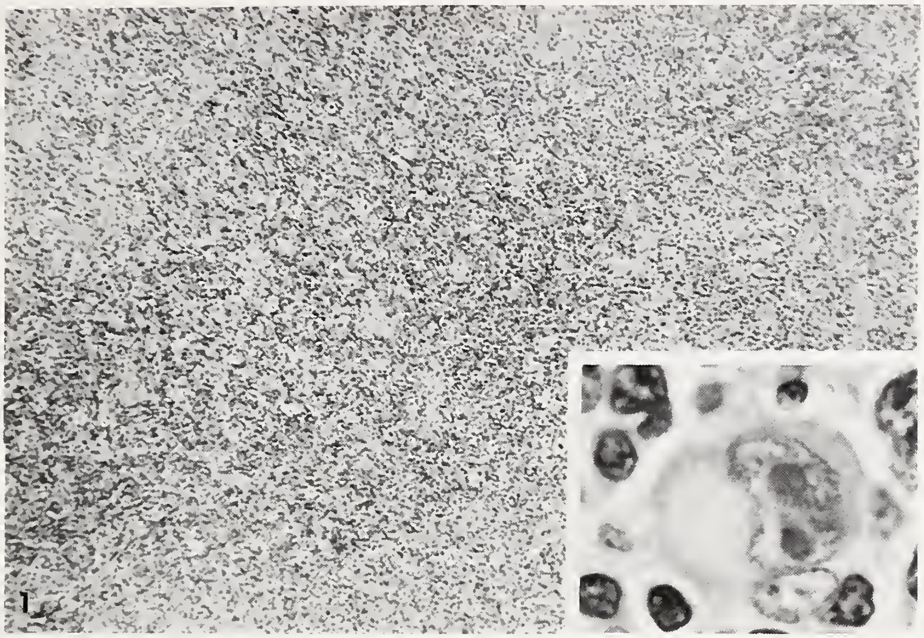


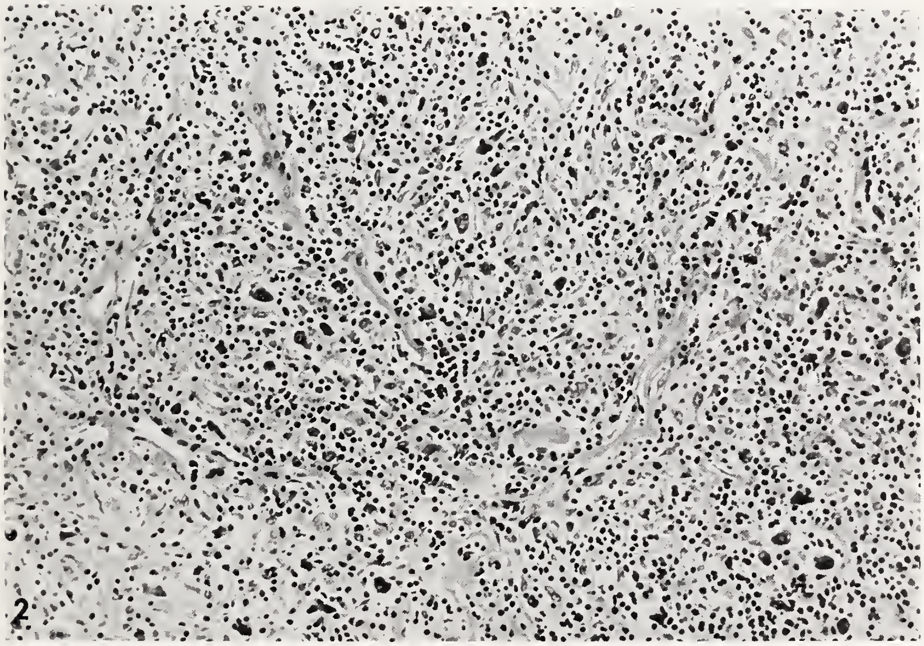
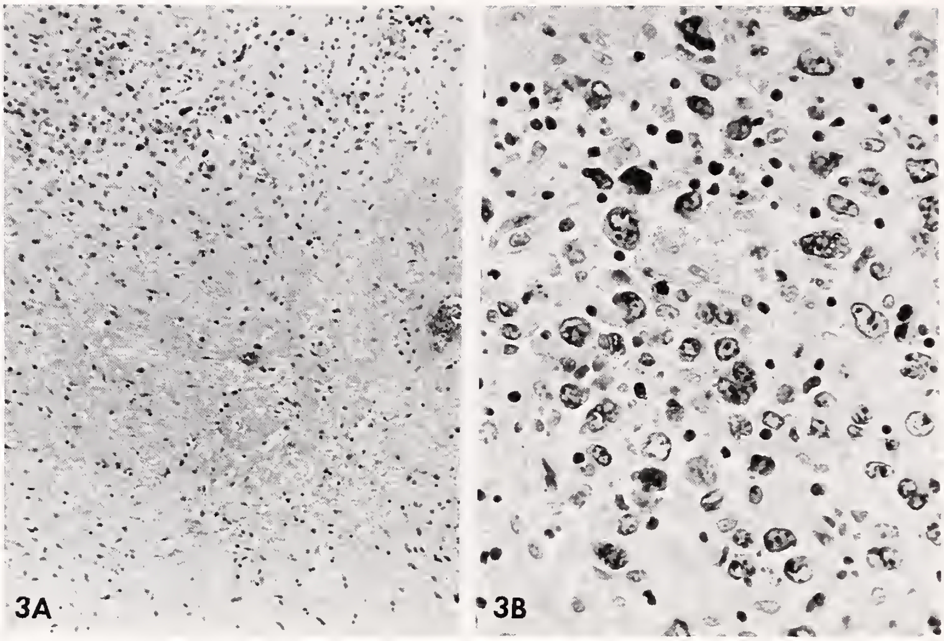


FIGURE 1.—LP. Diffuse proliferation of lymphocytes throughout node, mingled with mononuclear histiocytes and Reed-Sternberg cells (*inset*). × 120; *inset* × 2000

FIGURE 2.—MC. Increased number of Reed-Sternberg cells and decreased number of lymphocytes in conjunction with fibrosis. × 200



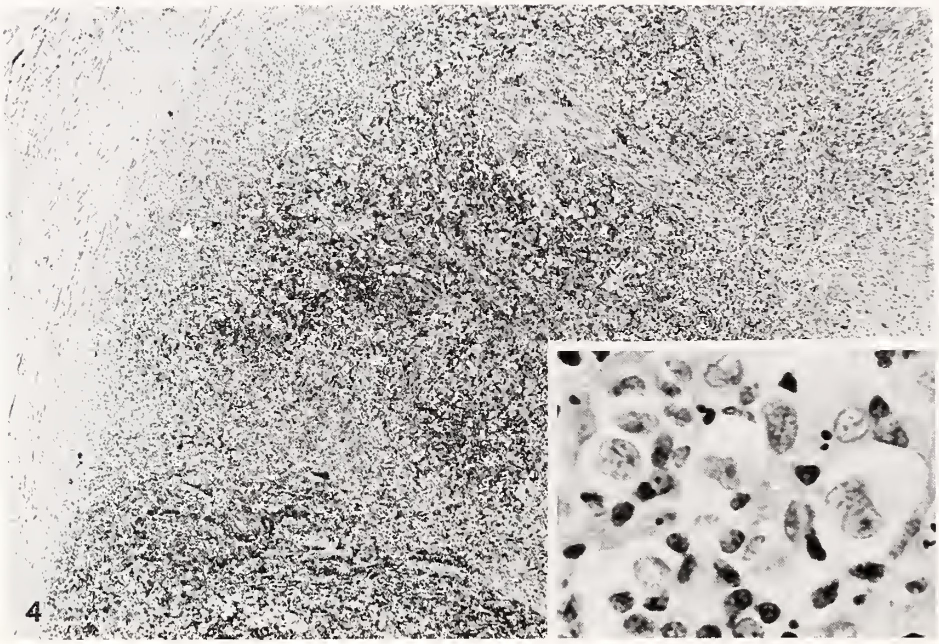


Figure 3.—LD. Decreased number of lymphocytes in diffuse fibrosis (3A) and reticular types (3B). × 120 (3A); × 800 (3B)

Figure 4.—NS. Bundle formation of collagen and appearance of lacunar cells (*inset*). × 80; *inset* × 800

Epidemiology and Histology of Hodgkin's Disease in Uganda[1]

D. H. Wright,[2] *Professor of Morbid Anatomy and Experimental Pathology, The University of Southampton, Southampton, England*

SUMMARY—Cases of Hodgkin's disease recorded in the Kampala Cancer Registry for the 5-year period 1964–68, inclusive, were analyzed histologically and epidemiologically. The age-standardized rates for males and females were far below those reported from Europe, North America, and Ibadan. This is to some extent due to underreporting, but the rate for Kyadondo County, an area with good medical services and communications, was still only half that for the United Kingdom and Ibadan. There appeared to be no geographic variation of Hodgkin's disease within Uganda, other than that due to variations in the quality of medical services. Hodgkin's disease in Uganda shows a shift toward the younger age groups, especially in males. When the incidence was calculated as age-specific rates, and allowance was made for underreporting, the incidence in Ugandan males probably exceeded that in British males for the first decade of life, but Ugandan males did not show an incidence peak in young-adult life and only a small peak in the fifth and sixth decades. Those histologic subtypes with poor prognosis predominated among the Ugandan patients. This was more marked in adults than in children. The nodular sclerosis subtype was relatively less common at all ages compared with series reported from the United States, but not with several series reported from the United Kingdom.—Natl Cancer Inst Monogr 36: 25–30, 1973.

THE POSSIBLE association of infectious agents with the etiology of Hodgkin's disease has sharpened interest in the epidemiology of this neoplasm. Correa and O'Conor (*1*) recently postulated that there are 3 and possibly 4 epidemiologic patterns of Hodgkin's disease, varying with the socioeconomic standards of the community. Pattern I is characterized by high rates in children and a preponderance of those histologic subtypes associated with a poor prognosis. This pattern is seen in Colombia. Pattern III, seen in urban communities in Europe and North America, is characterized by high rates in young adults and a predominance of those histologic subtypes associated with a good prognosis. Pattern II is intermediate between these 2 and is observed in rural communities in Europe and parts of North America. A fourth pattern characterized by low incidence rates at all ages may occur in some Asian countries, notably Japan.

High annual incidence rates for Hodgkin's disease in children were reported from various South American and African countries (*2, 3*), and it was noted that proportionally more children were affected by Hodgkin's disease in Uganda than in Europe or North America (*4*). Burn et al. (*5*) reported 133 East African children with Hodgkin's disease, but since these cases were drawn from an undefined population, incidence rates could not be calculated. All 3 reports on Hodgkin's disease in East Africa have noted the relative predominance of those histologic subtypes that carry a poor prognosis (*4–6*).

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Previously Reader in Pathology, Makerere University College Medical School, Kampala, Uganda.

The survey reported here of Hodgkin's disease in Uganda is based on material from the Kampala Cancer Registry. Since this serves a defined population, total and age-specific incidence rates can be calculated. The 1969 Uganda population-census figures were used for this purpose.

MATERIALS AND METHODS

The Kampala Cancer Registry was established in 1954 by Davies and Wilson (*7*). In 1960, the Department of Pathology of Makerere University College Medical School took over the diagnostic histopathology service for the whole of Uganda and provided a uniform service of good quality. All malignancies diagnosed between 1964 and 1968 inclusive were recently surveyed, and this report stems from that survey.

Paraffin-embedded tissue blocks were available for study. Sections were cut and stained by hematoxylin and eosin, Gordon and Sweet's method for reticulin, and by methyl green-pyronine. Other stains were used when indicated. Cancer Registry cards, available for each case, detailed the age, sex, home address, and tribe of the patient. A brief clinical history abstracted from the biopsy request form, or sometimes from the hospital case records, was written on the card. In most instances, this history was sufficient to indicate the main presenting clinical features, but was obviously totally inadequate to stage the disease. Follow-up information on most cases was nonexistent.

Sections were examined and classified according to the scheme recommended by the Nomenclature Committee at the Rye Conference on Hodgkin's Disease (*8*).

RESULTS

Tissue blocks and cards were available from 166 cases classified as Hodgkin's disease. Three of these were excluded because the tissue sections were of insufficient quality for a definite diagnosis. Thirty-two other cases were assigned to disease categories other than Hodgkin's disease (table 1). This represents a 20% misdiagnosis rate in the original series, a figure somewhat inflated by inclusion of cases in which Hodgkin's disease was given as a possible or tentative diagnosis. A similar high rate of misdiagnosis of cases of Hodgkin's disease was reported by Symmers (*9*). These figures indicate the need for caution when one analyzses "raw" incidence data for this disease.

TABLE 1.—*Diagnosis given to 32 cases originally diagnosed as Hodgkin's disease*

Disease	Number of cases
Reactive lymphadenopathy	8
Anaplastic metastatic tumor	9
Histiocytic lymphoma (reticulum cell sarcoma)	7
Lymphocytic lymphoma	4
Metastatic amelanotic melanoma	2
Myeloid metaplasia in lymph node	1
Hypocellular lymph node, ? cause	1
Total	32

Tables 2 and 3 show the age-standardized rates for Hodgkin's disease for males and females, by use of standardized European, world, and African populations. The rate for Uganda as a whole was very low when compared with rates of Ibadan, Birmingham, and Scotland. This low rate was probably partly related to the poor communications and lack of medical facilities in many areas of Uganda. Kyadondo, an area around Kampala, has the best medical facilities in the country. The rate for this district was 3 times that for Uganda as a whole, but was still only approximately half the rate for Ibadan, Birmingham, and Scotland.

TABLE 2.—*Age-standardized rates: Males*

	Europe	World	Africa
Uganda: All areas (1964–68: 5 yr: 100 cases)	0.5	0.5	0.5
Uganda: Kyadondo (1964–68: 5 yr: 12 cases)	1.5	1.4	1.5
Nigeria: Ibadan* (1960–65: 6 yr: 39 cases)	3.7	3.2	2.5
United Kingdom: Birmingham* (1963–66: 4 yr: 276 cases)	2.9	2.7	2.8
United Kingdom: Scotland* (1963–66: 4 yr: 270 cases)	2.9	2.5	2.3

* From: Cancer Incidence in Five Continents (*3*).

TABLE 3.—*Age-standardized rates: Females*

	Europe	World	Africa
Uganda: All areas (1964–68: 5 yr: 33 cases)	0.2	0.2	0.2
Uganda: Kyadondo (1964–68: 5 yr: 4 cases)	0.7	0.6	0.5
Nigeria: Ibadan* (1960–65: 6 yr: 15 cases)	3.0	2.4	1.6
United Kingdom: Birmingham* (1963–66: 4 yr: 176 cases)	1.8	1.5	1.5
United Kingdom: Scotland* (1963–66: 4 yr: 196 cases)	1.8	1.5	1.4

* From: Cancer Incidence in Five Continents (*3*).

TABLE 4.—*Hodgkin's disease rates (per million per yr) by use of a standardized Ugandan population in districts of Uganda, 1964–68*

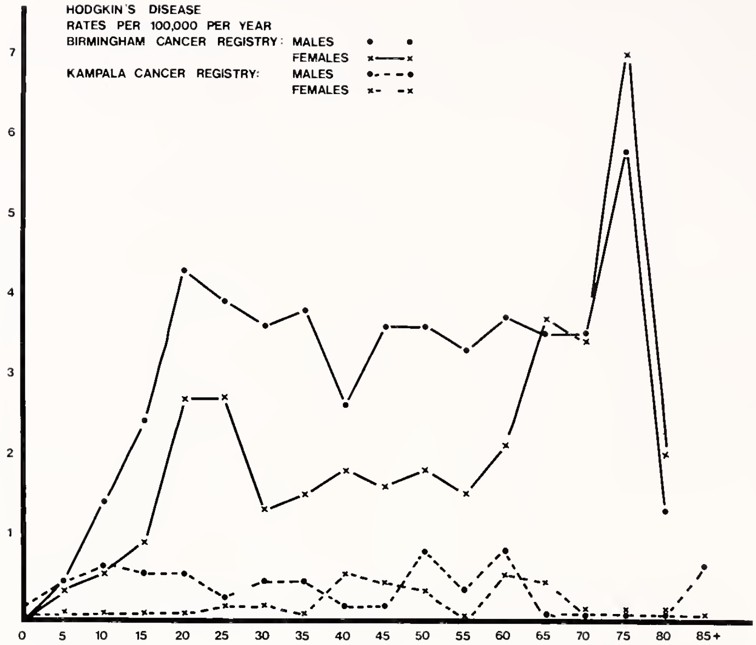
District	Males	Females
Acholi	5.0	2.7
Ankole	3.2	0.5
Bugisu and Sebe	2.0	1.9
Bukedi	0.8	0.8
Bunyoro	4.5	0.0
Busoga	4.0	1.7
Karamoja	1.7	0.0
Kigezi	4.8	0.5
Lango	7.2	1.0
Madi	0.0	0.0
Masaka	4.3	2.3
East Mengo	7.5	3.7
West Mengo	7.3	2.9
Mubende	2.9	0.0
Teso	7.3	0.5
Toro	4.8	0.0
West Nile	2.0	2.3
Uganda	4.6	1.5
Kyadondo	14.3	4.9

TABLE 5.—*Hodgkin's disease—Uganda, 1964–68*

Age (yr)	Male	Female	N/K	Total	Male/female
Unknown	0	1	1	2	0:1
0– 4	5	0	0	5	5:0
5– 9	17	4	0	21	4.2:1
10–14	18	3	0	21	6:1
15–19	10	2	0	12	5:1
20–24	8	1	0	9	8:1
25–29	5	3	0	8	1.6:1
30–34	7	2	0	9	3.5:1
35–39	7	1	0	8	7:1
40–44	2	4	0	6	0.5:1
45–49	1	3	0	4	0.3:1
50–54	6	2	0	8	3:1
55–59	2	0	0	2	2:0
60–64	4	2	0	6	2:1
65–69	0	1	0	1	0:1
85+	1	0	0	1	1:0
"Adult"	4	4	0	8	1:1
Total	97	33	1	131	3.3:1

Table 4 shows the age-standardization rates, by use of a standard Ugandan population, for each district of Uganda. The expected number of cases for each district was calculated on the basis of population and the incidence rate for Uganda as a whole. Discordance between observed and expected cases appeared, in general, to be explicable on the basis of variation in the quality of medical services between the districts. This was further tested by use of the incidence rate for all cancers in each district to calculate the expected number of cases of Hodgkin's disease. These figures demonstrated that the distribution of Hodgkin's disease within Uganda appears to be determined by the quality of medical services and communications and is not related to climatic parameters associated with the distribution of Burkitt's lymphoma.

Table 5 shows the age and sex distribution of the Ugandan patients with Hodgkin's disease. In comparison with the age distribution seen in Europe and North America, there was a marked relative shift toward the younger age groups. However, when the age-specific incidence rates were plotted and compared with those from the Birmingham Cancer Registry (text-fig. 1), only in the first 5 years of life did the incidence rate of Ugandan males equal that in



TEXT-FIGURE 1.—Comparison of age-specific incidence rates of Ugandan patients with Hodgkin's disease with those of cases from the Birmingham Cancer Registry.

Birmingham. By the age of 10, the Ugandan incidence was half that seen in Birmingham. Thereafter, the Ugandan incidence leveled off and did not show the steep rise seen in males in the second decade of life in Europe and North America. After the age of 20, the incidence in Ugandan males remained at approximately one-sixth the incidence for males recorded in the Birmingham Cancer Registry. The incidence in females in Uganda remained very low throughout all age groups.

The number of cases of Hodgkin's disease in Kyadondo County was too small for a meaningful age distribution to be plotted. However, the age distribution in those areas of central Uganda, with relatively good communications and medical services, did not differ significantly from that of Uganda as a whole.

The sex incidence (table 5) showed a marked male preponderance in the first 2 decades and approached unity thereafter with an overall male-to-female ratio of 3.3:1.

TABLE 6.—*Main presenting site of tumor*

Site	Number of tumors
Lymph nodes:	
Cervical	50
Mediastinal	1
Axillary	3
Retroperitoneal	2
Inguinal	12
Multiple	11
Generalized	33
Unspecified	2
Anterior thorax	1
Liver	5
Spleen	4
Retroperitoneum (unspecified)	1
Intestine	1
Bone	1
Unspecified	4
Total	131

The main presenting features of the tumor are shown in table 6. The predominance of superficial lymphadenopathy as a presenting feature and relative lack of visceral and deep-seated lesions were related to the unsophisticated medical investigations carried out in most cases and the relative ease of biopsy of the superficial lymph nodes. There was no apparent increase in inguinal adenopathy associated with the habit of walking barefoot.

Histologic typing was not possible in 3 cases because of the smallness of the biopsy. The modified classification recommended by the Nomenclature Committee at the Rye Conference on Hodgkin's Disease (*8*) was used to type the remaining 128 cases. The subdivision into the different histologic types is shown in table 7 and is shown for each age group in table 8. There was an overall deficiency in the nodular sclerosis (NS) subtype when compared with series reported from the United States (table 9). Several series reported from the United Kingdom also showed a deficiency of NS when compared with the U. S. reports.

TABLE 7.—*Classification of 128 cases of Hodgkin's disease*

Histologic type*	Male	Female	Not known	Total	Male: female ratio
LP	20	3	—	23	6.7:1
NS	11	4	—	15	2.7:1
MC	46	18	1	65	2.6:1
LD	19	6	—	25	3.1:1
Total	96	31	1	128	3.1:1

* Histologic typing not possible in 3 cases.

TABLE 8.—*Histologic type* for each age group*

Age (yr)	LP	NS	MC	LD	Total
0–4	1	0	4	0	5
5–9	4	4	11	1	20
10–14	7	1	10	2	20
15–19	1	3	5	3	12
20–24	2	2	4	1	9
25–29	0	1	4	3	8
30–34	0	0	4	5	9
35–39	1	1	5	1	8
40–44	0	0	5	1	6
45–49	1	0	2	1	4
50–54	1	0	4	3	8
55–59	1	1	0	0	2
60–64	3	0	1	2	6
65–69	0	0	1	0	1
85+	0	0	1	0	1
Adult	1	1	3	2	7
Not known	0	1	1	0	2
Total	23	15	65	25	128

* Not classified, 3.

TABLE 9.—*Histologic classification*

Author	Country	Number of cases	Age	Histologic type LP (%)	NS (%)	MC (%)	LD (%)
Present series	Uganda	128	All ages	18	12	50	20
Olweny et al., 1971 (*6*)	Uganda	100	Adults	8	7	47	38
Burn et al., 1971 (*5*)	East Africa	133	15 yr	19.5	10.5	31.6	38.4
Burn et al., 1971 (*5*)	United Kingdom	53	15 yr	30.2	11.3	49.1	9.4
Gough, 1970 (*14*)	United Kingdom	96	All ages	20	15	29	36
Farrer Brown et al., 1971 (*16*)	United Kingdom	50	All ages	14	56	30	0
Lukes and Butler, 1966 (*11*)	United States	377	All ages	16	40	26	18
Kadin et al., 1971 (*10*)	United States	117	All ages	11	73	16	0

The Ugandan patients showed a deficit of lymphocyte predominance (LP) and an excess of mixed cellularity (MC) and lymphocyte depletion (LD) when compared with series reported from the United Kingdom and the United States. This paucity of cases showing LP was most marked in the age groups >15 years old as was also the excess of cases showing LD. Sarcoid-like collections of non-neoplastic histiocytes were prominent in only 4 cases in the series. Such collections were seen much more frequently in Hodgkin's disease from other areas of the world (*10*). Their relative infrequency in the Ugandan material was partly due to the paucity of cases showing LP, but it might be of significance that sarcoidosis itself is uncommon in Uganda.

DISCUSSION

The polymorphic histologic appearance of Hodgkin's disease creates difficulties in diagnosis; Symmers (*9*) discussed the high rate of misdiagnosis that can occur with this disease. In the present survey, 20% of cases, originally included under the diagnosis of Hodgkin's disease or possible Hodgkin's disease, were excluded after careful study and placed in other histologic categories (table 1). One-half of these cases, i.e., those subsequently diagnosed as anaplastic metastatic tumor, histiocytic lymphoma, and amelanotic melanoma, would, if included in the survey, have been diagnosed as LD Hodgkin's disease and have distorted the analysis in favor of this histologic subtype. This high rate of misdiagnosis is not unique to Uganda and should be taken into consideration when one analyzes incidence rates based on "crude" cancer registry data.

The age-standardized rates for Hodgkin's disease in the whole of Uganda were in the region of one-sixth the rate reported from Ibadan and the United Kingdom. The rate in Kyadondo County was 3 times that for Uganda as a whole, but still only half the rate for Ibadan and the United Kingdom. Undoubtedly the low incidence for Uganda as a whole was due partly to underreporting associated with the poor medical services in large areas of the country. However, such reservations probably do not apply to Kyadondo County. Davies et al. (*11*), in a survey of all types of cancer in Uganda, noted that the total cancer incidence rates for Kyadondo were in general of the same order as those of Denmark for the population between ages 1 and 45 years. It would appear, therefore, that throughout Uganda the age-standardized incidence rate for Hodgkin's disease was substantially less than that in Ibadan and the United Kingdom, and that this was only partly due to underreporting of cases.

The proportion of Ugandan patients <15 years old with Hodgkin's disease (47/131) was higher than that seen in developed countries. This was largely due to the difference in the age structure of the population of Uganda compared with that of Europe and North America which have relatively aged populations. When age-specific incidence rates were calculated, only in the first 5 years of life did the rate in Ugandan males exceed that in British males (text-fig. 1). Ugandan males did not show the steep rise in incidence between 5 and 15 years shown by British males. There was no peak in young-adult life and only a very small peak in the fifth and sixth decades.

It has been shown that the age distribution of Hodgkin's disease is similar in those areas of central Uganda with relatively good medical services to the distribution in Uganda as a whole. Thus, it can be assumed that underreporting in remote districts does not selectively affect any age group <60 years old. If it is assumed that the rate for Kyadondo County is the true incidence rate and not grossly affected by underreporting, the figures for the rest of Uganda would need to be multiplied by a factor of approximately 3 to compensate for underreporting. The application of such a factor to text-figure 1 gave an incidence for males similar to that reported by Correa and O'Conor (*1*) in Colombia in that there was a high incidence in childhood but no peak in early adult life. The incidence graph would, however, differ from that seen in Colombia and most other areas of the world because it would not show the usual pronounced peak seen in the fifth and sixth decades.

There was a very low incidence of Hodgkin's disease in females in Uganda at all ages. In comparison with the males, this was most marked in the first 15 years of life, when the male-to-female ratio was 5.7:1. Fraumeni and Li (*12*) noted a similar marked male excess in childhood Hodgkin's disease in the United States. Even if a threefold factor was allowed for the degree of underreporting in Uganda, the incidence in Ugandan females would remain below that in British females at all ages.

Previous investigators have reported a relative excess in Africans of the MC and LD histologic subtypes of Hodgkin's disease (*4–6*). Correa and O'Conor (*1*) noted a similar excess of these subtypes in Colombia. There was a striking deficiency of the NS subtype in Uganda in comparison with the United States (*13*), though not in comparison with the British series reported by Burn et al. (*5*) and Gough (*14*) (table 9). The excess of those histologic subtypes with a poor prognosis was not directly related to the age distribution of the Ugandan cases. When patients <15 years old were compared with those >15 years old, the LP subtype occurred in 27% of the former and 14% of the latter. Conversely, the LD subtype occurred in only 7% of the <15-year-old group and 27% of the >15-year-old group. The relative incidence of NS and MC was the same in both age groups (table 8).

Correa and O'Conor (*1*) postulated a relationship between socioeconomic status, age susceptibility, and host response in Hodgkin's disease. In communities with poor socioeconomic standards, exposure to the hypothetical etiologic agent occurs at an early age, giving rise to a high incidence of the disease in male children who, because of an immature or defective host response, develop those histologic subtypes of Hodgkin's disease that have a poor prognosis. They noted, however, that most young adults who developed the disease in Colombia also displayed the less favorable subtypes of MC and LD. In more highly developed countries, children are protected from infectious disease, and exposure to the agent or agents of Hodgkin's disease does not occur until young-adult life, when the better host response is reflected in the predominance of histologic subtypes with a favorable prognosis.

In any analysis of the epidemiology of Hodgkin's disease in Uganda, in comparison with Europe and North America, consideration must be given to the high incidence of infective disease in Africa. Holoendemic malaria, in particular, has a profound influence on the population. Repeated exposure to the malarial parasite from birth results in either death or immunity by the early teens. Thus, there is a considerable culling of the population, and the survivors are both selected and have a profoundly stimulated and altered lymphoreticular system. Such a background might well be expected to influence the incidence and behavior of Hodgkin's disease, known to be associated in some instances with impairment of cell-mediated immunity.

Possibly the age distribution of Hodgkin's disease in Uganda is related to early exposure to one or more infectious etiologic agents. The suggestion that this early exposure leads to the development of those histologic subtypes of Hodgkin's disease with a poor prognosis (*1*) does not hold for Uganda, since there were more cases with LD and fewer with LP among the adults than among the children. The overall low incidence of Hodgkin's disease in Uganda could be related to the elimination of a susceptible population by infective disease or due to the fact that the surviving population, by virtue of exposure to infective disease, has an altered lymphoreticular system less susceptible to the development of Hodgkin's disease. These suggestions do not account for the reported high incidence of Hodgkin's disease in Ibadan.

An intriguing hypothesis was recently put forward: The features of Hodgkin's disease can be explained by viral transformation of thymus-derived cells leading to the cell-mediated autoimmunity, thymus-derived cell depletion, and eventual neoplasia (*15*). Previous infection and stimulation of the lymphoreticular system would be expected to profoundly influence such a system, and it might be of relevance, in view of the low incidence of Hodgkin's disease in Uganda, that autoimmune disease in general is uncommon in tropical Africa.

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Hodgkin's Disease in Western Europe: A Review [1,2]

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SUMMARY—A review of published data showed no good evidence for geographic differences in the epidemiology of Hodgkin's disease within Western Europe. The age distribution was bimodal, with a first peak between 20 and 30 years of age and a second peak at ages after 50 years. There was no obvious difference in the sex ratio at the first and second modes. There was no evidence that the incidence peak in young adults occurred at different ages in different parts of Western Europe. All histologic types, according to the Rye classification, appeared to have a bimodal age distribution, but the relative heights of the incidence peaks differed for the different types. There was some evidence of a higher incidence in rural than in urban areas in male children. Time-space clustering was suspected in some studies, but remains unproved.—Natl Cancer Inst. Monogr 36: 31–36, 1973.

ACCORDING TO published incidence and mortality rates, Hodgkin's disease appears to be the type of neoplasm of hematopoietic and lymphatic tissues that varies least among the countries of Western Europe, and the rates are similar to those in the United States and Canada (*1–5*). Studies on time trends indicate either that the frequency of Hodgkin's disease has not changed during the last several decades, or that recorded rates have modestly increased which could easily be explained by improvements in diagnostic services (*1, 6–10*).

The age-specific incidence rates showed a bimodal distribution that varied little from one cancer registry to another within Western Europe (*2*). Text-figure 1 presents the age distributions for Denmark, Finland, Norway, and Sweden, all of which have national cancer registries covering the total population in the respective countries. Both for males and females, there was a first peak between 20 and 30 years of age and slightly lower rates in the thirties and early forties; thereafter, the rates climbed to a level of about 2 times that of the first peak. A similar bimodal pattern is seen in data from the 4 largest British registries (text-fig. 2) and in data reported from a series of regional surveys and hospital materials (*2, 10–19*) and is also apparent from mortality statistics (*3, 6, 8, 15, 20, 21*).

Incidence curves were compared with mortality curves (text-fig. 3) for the combined data from the Fenno-Scandinavian countries (Denmark, Finland, Norway, and Sweden), for which national data on both incidence and mortality are available (*2, 3*), and for England and Wales, for which the national mortality rates (*3*) have been compared to the combined incidence rates from 5 regional registries (Birmingham, Oxford, Sheffield, South Western Region, and Liverpool) (*2*). The combined age-specific rates are the unweighted means of age-specific rates from each country or region. For both sexes and in both geographic areas, incidence rates were substantially higher than mortality rates in individuals <40 years old. At later ages, the difference

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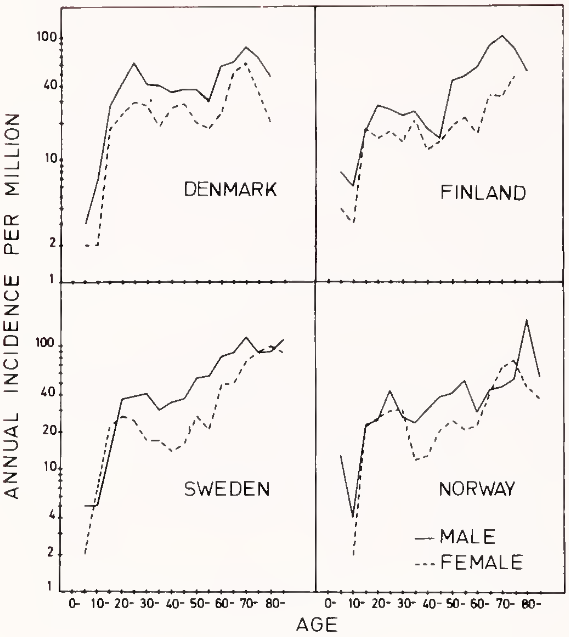
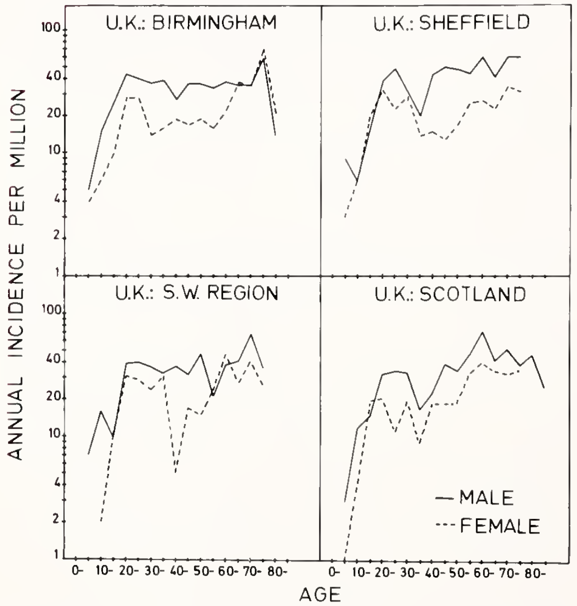
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

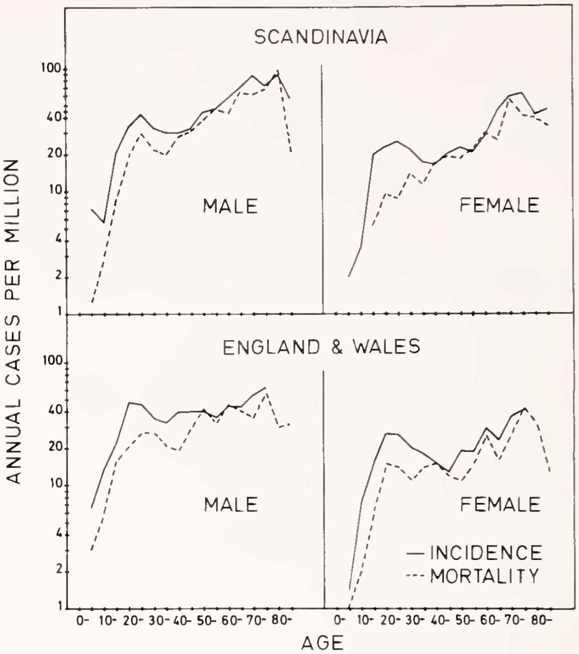
[2] Supported by Public Health Service contract PH43-64-499.



Text-figure 1.—Age-specific incidence rates for Hodgkin's disease in Fenno-Scandinavian countries. Data from Doll et al. (*2*).



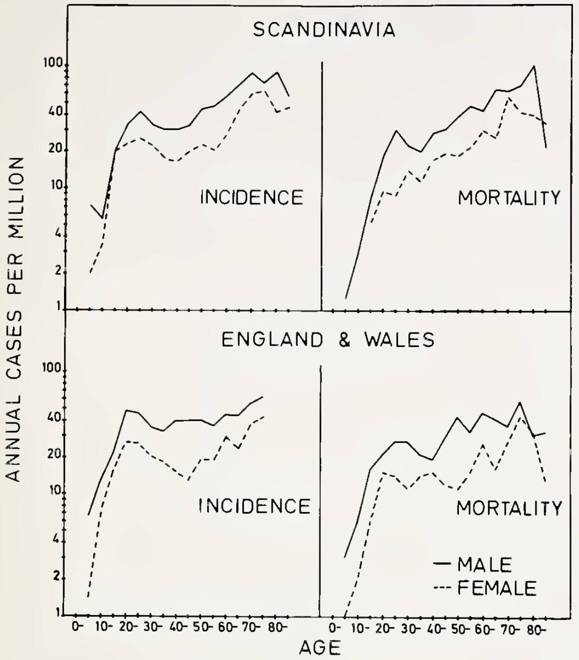
Text-figure 2.—Age-specific incidence rates for Hodgkin's disease in 4 regional cancer registries of the United Kingdom. Data from Doll et al. (*2*).



Text-figure 3.—Age-specific incidence and mortality rates for both sexes in Scandinavia (Denmark, Finland, Norway, and Sweden) and in England and Wales. Data from Doll et al. (*2*) and the World Health Organization (*3*). The plotted rates for Scandanavia are the unweighted means of age-specific rates for each country. Incidence rates for England and Wales are the unweighted means of age-specific rates from the cancer registries of Birmingham, Oxford, Sheffield, South Western Region, and Liverpool. Sex ratio is expressed by the vertical distance between the frequency curves for males and females.

between mortality and incidence rates became much smaller.

A difference in the sex ratio at 15–35 years of age as compared to that at ages >50 years was one of the arguments behind the hypothesis of MacMahon (*22*) that the first and second incidence peaks belonged to 2 different disease entities. In 4 of 5 large incidence surveys, he noted a sex ratio close to 1.0 during the first mode and closer to 2.0 during the second mode. Neither the combined Fenno-Scandinavian incidence rates nor the combined incidence rates from English registries (text-fig. 4) showed any such variation in sex ratio with age. As MacMahon also noted (*22*), mortality rates have about the same sex ratio at all ages >15 years. In children, a stronger male preponderance has



TEXT-FIGURE 4.—Same data as in text-figure 3, plotted for the comparison of incidence and mortality rates.

been assumed, but evidence conflicts on this point (*10*).

It has been suggested that the first peak of the frequency curve occurs at an earlier age in some parts of the world than in others (*23*). In a search for possible differences in the position of the adolescence peak in different countries in Western Europe, the cumulative incidence of Hodgkin's disease at ages 10–24 years was calculated as a percentage of the cumulative incidence at ages 10–39 years (table 1); similar calculations were made for mortality (table 2). The calculations were made from age-specific rates (*2, 3*) and European standard population (*2*). These data did not indicate any systematic difference between different parts of Western Europe or between Western Europe and North America.

In a recent review of the histologic material from all lymphomas diagnosed in Norway during 1 calendar year (*1*), 115 cases were recognized as Hodgkin's disease. When typed according to the Rye classification, mixed cellularity (MC) was the most frequent type and accounted for 33% of the total, followed by nodular sclerosis (NS) 29%, lymphocytic depletion (LD)

TABLE 1.—*Cumulative incidence of Hodgkin's disease at ages 10–24 years, in percent of cumulative incidence at ages 10–39 years, calculated from age-specific incidence rates and European standard population (2)**

Male		Female	
Sweden	34	Denmark	36
Denmark	35	Norway	41
Norway	36	Finland	41
Finland	41	United Kingdom	45
United Kingdom	42	Sweden	49
United States	35	Canada	43
Canada	42	United States	53

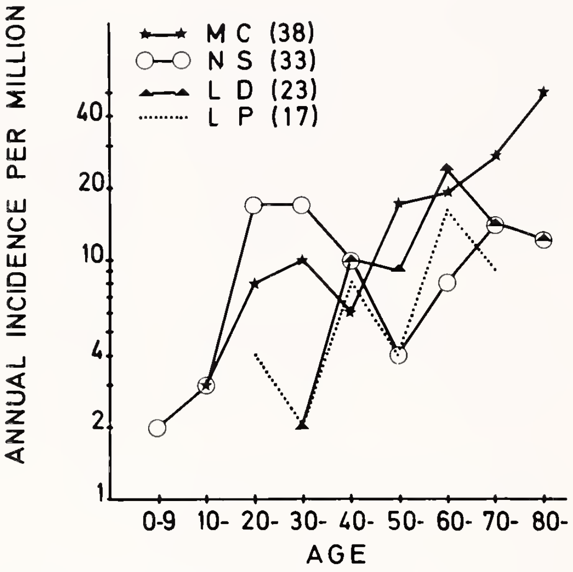
* USA: mean value for Alameda whites and Nevada. Canada: mean value for Alberta, Manitoba, Quebec, and Saskatchewan. United Kingdom: mean value for Birmingham, Oxford, Sheffield, South Western Region, Liverpool, and Scotland.

TABLE 2.—*Cumulative mortality from Hodgkin's disease at ages 10–24 years, in percent of cumulative mortality at ages 10–39 years, calculated from age-specific mortality rates (3) and European standard population (2)*

Male		Female	
Scotland	17*	Finland	15†
Italy	25	France	17
France	25	Greece	20*
Austria	27*	Sweden	20*
Finland	27*	The Netherlands	23
Denmark	27*	German Federal Republic	24
Belgium	28	Austria	26*
Sweden	28*	Norway	26†
The Netherlands	32	Belgium	32*
Greece	33*	Italy	35
Switzerland	33	Switzerland	36*
German Federal Republic	33	England and Wales	37
England and Wales	37	Scotland	53*
Norway	43†	Denmark	58†
United States	27	United States	31
Canada	32	Canada	42

* Calculated from <30 cases at ages 10–39 years.
† Calculated from <10 cases at ages 10–39 years.

20%, lymphocytic predominance (LP) 15%, and unclassified 3%. The age-specific incidence rates for each histologic type are shown in text-figure 5. The MC type was bimodal, and the second peak was substantially higher than the first peak. NS was also apparently bimodal, but the second peak was not quite as high as the first peak. LD was not diagnosed in individuals <35 years old in this material, and the LP type was also mainly found in the older age groups.



Text-figure 5.—Age-specific incidence rates for the histologic types of Hodgkin's disease in Norway, 1968. Total number of patients in each group is shown in parentheses.

To my knowledge, no other population-based data on the distribution of the histologic types of Hodgkin's disease according to the Rye classification have been published from Western Europe. Studies based on material from single hospitals or institutes of pathology have been published from Finland by Franssila et al. (*16*), from the German Federal Republic by Hamann et al. (*17*), and from England by Smithers (*18*). All these materials agree that the MC type shows a bimodal age distribution. The larger materials of Smithers and Hamann et al. also indicate that the LD and the LP types are both bimodal. NS at first sight may appear to be unimodal because it is presented in these studies by the number of cases in each age group. The impression gained from the published diagrams is that of a high peak at 20–30 years of age, with gradually decreasing frequencies thereafter. However, this appears to be a false picture produced by a selection of younger patients for admission to specialized cancer treatment centers and by the disregard of the smaller size of the population at risk in the older age groups. The relative frequency of NS after 50 years of age, as related to Hodgkin's disease of all types, was even higher in the hospital materials than in the Norwegian population-based material (table 3), and MacMahon et al. (*24*) estimated from Smithers' data that the incidence rate for NS at ages >50 years was not much lower than that between 20 and 30 years, similar to the findings in the Norwegian population (*1*). In the material of Hamann et al. (*17*), from a large general pathology unit that mostly covers a defined region of the German Federal Republic, a low second mode of NS after the age of 50 years was seen in both sexes. Most likely, from the available data on histologic types of Hodgkin's disease in Western Europe, all 4 histologic types share in the bimodal age-distribution pattern, but the relative height of the first and second incidence peaks differs for each histologic type. The incidence of NS is higher between 20 and 30 years than after 50 years, but the difference is not very large. The incidence of MC is more than twice as high after 50 years as it is between 20 and 30 years. The incidences of the LP and the LD types are several times higher after 50 years of age than at 20–30 years.

Table 3.—*NS in percent of all histologically classified cases of Hodgkin's disease in 4 European studies*

Authors	Age (yr)					
	0–19	–29	–39	–49	–59	60–
Franssila et al. (*16*)*	53	55	55	42	16	32
Hamann et al. (*17*)*	41	54	45	37	28	28
Smithers (*18*)*	65	53	51	36	30	23
Stalsberg (*1*)	60	60	54	29	13	16

* Approximate figures calculated from published graphs.

In Northern Germany, the mortality from Hodgkin's disease in individuals <20 years old

TABLE 4.—*Annual incidence per 100,000 of Hodgkin's disease at ages 0–24 years in urban and rural areas. Data from (15, 25–27)*

	Male (yr)					Female (yr)				
	0–4	–9	–14	–19	–24	0–4	–9	–14	–19	–24
Denmark 1943–57										
Urban*	0.1	0.2	0.3	2.2	4.5	0.0	0.1	0.4	1.1	2.3
Rural	0.2	0.6	0.7	2.1	3.3	0.1	0.1	0.3	1.6	3.5
Finland 1963–67										
Urban	0.0	0.5	0.5	1.9	3.7	0.2	0.5	0.8	1.8	1.4
Rural	0.2	1.1	0.4	0.9	2.4	0.0	0.2	0.2	1.0	0.9
Norway 1959–61, 1964–66										
Urban	0.0	0.3	0.3	1.0	2.5	0.0	0.0	0.4	3.4	2.0
Rural	0.0	1.3	0.3	2.4	3.1	0.0	0.0	0.4	1.5	2.3

* Capital and provincial towns.

appeared to be higher in rural areas than in urban areas *(21)*. A comparison of age-specific incidence rates in urban and rural areas in Denmark *(15)*, Finland *(25)*, and Norway *(26, 27)* (table 4) showed that only in males between 5 and 9 years of age were the incidence rates higher in rural than in urban areas in all 3 countries. In the lymphoma survey in Norway *(1)*, the urban/rural relationship differed markedly among the different histologic types of Hodgkin's disease. NS was nearly 3 times more frequent in urban than in rural areas, whereas the MC and LD types were more frequent in rural than in urban areas. Thus there is some evidence that the rural population develops the disease at a somewhat earlier age and tends to show the less favorable histologic types.

Clustering has been suspected, but never proved. There was some evidence of time-space clustering in early data from the Danish cancer registry *(28)*, but the evidence was too weak for any conclusions to be reached. Data from the Cancer Registry of Norway for a 10-year period *(10)*, when cross-classified by counties of residence and 2-year calendar periods of diagnosis, showed a statistically significant time-space clustering. However, further analysis of the data did not support the initial finding *(29)*. Data from the Manchester region *(19)* showed some evidence of time-space clustering among patients 15–44 years old in 2 calendar years, but the total material for all ages and a 7-year period did not support this evidence. Data from Northern Germany *(21)* showed no evidence of time-space clustering. However, the methods of analysis applied are not very powerful, and the last word has obviously not been said on the problem of clustering.

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Hodgkin's Disease in Israel [1,2]

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SUMMARY—During the 9-year period, 1960–68, the average annual incidence rate for Hodgkin's disease in Israel was 2.1/100,000 in males and 1.9/100,000 in females. The histologic sections of 371 cases of malignant lymphoma diagnosed throughout the country during a 2-year period were reviewed by one team of pathologists. Hodgkin's disease accounted for 27.5%, reticulum cell sarcoma for 34.5%, and lymphosarcoma for 33.2% of the cases reviewed. Hodgkin's disease was the predominant type in young adults, whereas reticulum cell sarcoma and lymphosarcoma predominated in the older age groups. The male: female ratio for Hodgkin's disease (1.17:1) was lower than that reported from most other countries. Sixteen (15%) of 107 cases registered as Hodgkin's disease in the Israel Cancer Registry during the 2-year period were reclassified as reactive hyperplasia, lymphosarcoma, reticulum cell sarcoma, or nonlymphoid malignancy, after review of the histologic sections. On the other hand, 11 of 289 cases registered as lymphosarcoma or reticulum cell sarcoma were reclassified as Hodgkin's disease. Nodular sclerosis (34%) and mixed cellularity (30%) were the most common types of Hodgkin's disease encountered. Mixed cellularity was the most common type in children and nodular sclerosis predominated in young adults. Preliminary data are presented on differences in the frequency and histologic subtypes of Hodgkin's disease among the various population groups in Israel.—Natl Cancer Inst Monogr 36: 37–44, 1973.

ISRAEL IS a small country with a population of just over 3 million, of whom 85% are Jews and 15% non-Jews (Moslems, Christians, or Druzes) (*1*). The Jewish population includes groups who have migrated to Israel from many different countries and who differ markedly in their demographic makeup and sociocultural characteristics.

It has recently been suggested that the varied epidemiologic patterns of Hodgkin's disease described in different communities can be explained on the basis of an interplay of environmental and host etiologic factors (*2*). It, therefore, seems of particular importance to determine whether different patterns of Hodgkin's disease exist among the various population groups in Israel. To answer this question, a country-wide survey of Hodgkin's disease and other malignant lymphomas is being planned within the framework of the recently established Irving H. Levin Center (Jerusalem) for the study of Hodgkin's disease. The following report on Hodgkin's disease in Israel is based on: 1) cancer registry data for 1960–68 and 2) preliminary results of a survey in which the histologic sections of all

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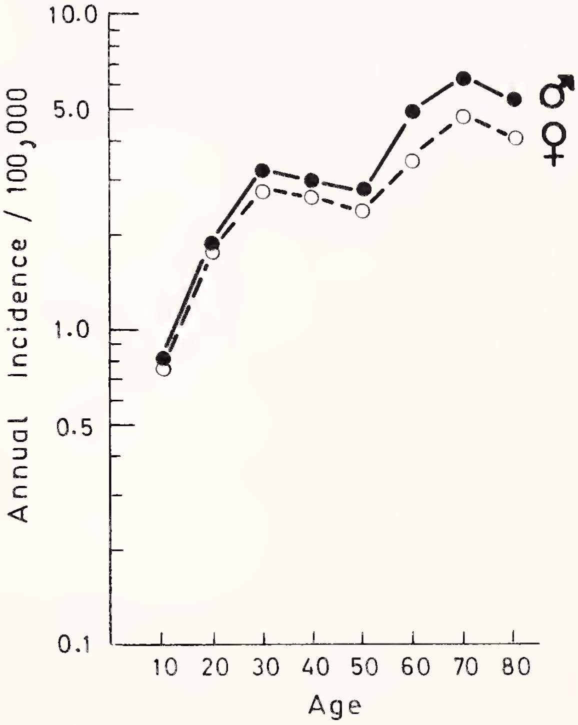
malignant lymphoreticular tumors diagnosed in pathology laboratories throughout the country during a 5-year period are being reviewed by one team of pathologists.

Age-Specific Rates for Hodgkin's Disease Based on Cancer Registry Data

The annual age-specific incidence rates for Hodgkin's disease per 100,000 of the population, as based on cancer registry data for 1960–68, are shown in text-figure 1. The Israel Cancer Registry operated by the Ministry of Health covers the whole population and receives information from hospital admissions, pathology departments, and death certificates. For the calculation of the incidence rates, population figures for 1960–68, as published by the Central Bureau of Statistics, were used (*3–6*). For the Jewish population, the denominator was the sum of the average population figures for each year and, for the non-Jewish population, the sum of the figures for the end of each year. For 1961, the census figures (May 1961) were used.



Text-figure 1.—Age-specific incidence rates for Hodgkin's disease, by sex. Cancer registry data, Israel, 1960–68.

During the 9-year period, January 1, 1960, to December 31, 1968, 468 new cases of Hodgkin's disease in residents of Israel were registered at the Israel Cancer Registry—257 in males and 211 in females. This represents an average annual incidence rate of 2.1/100,000 in males and 1.9/100,000 in females. The diagnosis was based on histologic examination in 89% of cases and on clinical or radiologic examination in 11%.

The rates in males rise from about 0.8/100,000 in children 5–14 years old to reach a peak of 3.3/100,000 in young adults 25–34 years old. There is then a leveling off or a slight drop over the next 2 decades. After the age of 50 years, the rates rise again to reach a peak of 6/100,000 in the 65- to 74-year-old group. The rates for male children are somewhat higher and those for young adults somewhat lower than those found in developed urban communities of Europe and the United States. This corresponds to what Correa and O'Conor have referred to as the "intermediate pattern" or pattern II, and is similar to that seen in rural Norway (*2*). The incidence curve for females has the same shape as that for males but is at a slightly lower level, especially in the older age groups. The slight differences between the rates in male and female children are unlike what has been reported from most countries.

We are reviewing the histologic sections of all cases of malignant lymphoma diagnosed in Israel during the 5-year period, 1964–68. This study is still in progress, but it is clear that the cancer registry figures for Hodgkin's disease include a certain percentage of cases which, on review, prove to be atypical hyperplasias, lymphoid malignancies other than Hodgkin's disease, or even nonlymphoid malignancies. On the other hand, exclusion of these cases is partly balanced by cases registered as lymphosarcoma, reticulum cell sarcoma, or malignant lymphoma, type unspecified but reclassified as Hodgkin's disease on review. Preliminary figures on the extent to which the rates for Hodgkin's disease based on cancer registry figures differ from those calculated after review of the histologic sections of all registered cases of malignant lymphoma will be discussed later in this report.

Relative Frequency of Hodgkin's Disease and Other Types of Malignant Lymphoma

We have recently completed our review of the histologic sections of all cases of malignant lymphoma diagnosed in Israel during the 2-year period January 1, 1967, to December 31, 1968 (7). All sections were reviewed independently by at least 2 pathologists; all controversial cases were examined by 3 pathologists who participated in the study, and a consensus was arrived at after a discussion.

The diagnosis of malignant lymphoma was confirmed in 371 (94%) of the 396 cases reviewed. Hodgkin's disease accounted for 27.5% of the cases, reticulum cell sarcoma for 34.5%, and lymphosarcoma for 33.2%. There were 8 cases (2.2%) of Burkitt-like lymphoma in children and 11 cases (2.7%) of "unclassified or other lymphomas."

The relative frequency of the 3 main histologic types of malignant lymphoma in the various age groups is shown in table 1. Hodgkin's disease predominated in young adults, whereas reticulum cell sarcoma and lymphosarcoma predominated in the older age groups. Hodgkin's disease accounted for 53% of all malignant lymphomas in young adults 15–34 years of age, but for <10% of those in individuals 65 years of age. These findings stress the importance ot taking the age structure of the population into account when one compares the relative frequencies of reticulum cell sarcoma, lymphosarcoma, and Hodgkin's disease in different ethnic and geographic groups.

The 102 cases of Hodgkin's disease diagnosed during the 2-year period included 55 males and 47 females. This male:female ratio of 1.17:1 was lower than that reported from most other countries. A similar low male:female ratio for Hodgkin's disease in Israel was found by Modan and his co-workers in their review of cases diagnosed during 1960–64 (*8*). The sex ratio varied in the different age groups, however. Thus, the 102 histologically confirmed cases included 8 cases in boys but only 3 cases in girls <15 years old. On the other hand, in young adults, there were more cases in females than in males, especially in the 15- to 19-year-old group in which 8 of 11 cases were in females.

TABLE 1.—*Histologically confirmed malignant lymphoma, Israel, 1967–68: Relative frequency of lymphosarcoma, reticulum cell sarcoma, and Hodgkin's disease in different age groups*

Age (yr)	Number of cases*	Lymphosarcoma (%)	Reticulum cell sarcoma (%)	Hodgkin's disease (%)
0–14†	38	31.6	10.5	28.9
15–34	68	23.5	23.5	53
35–49	60	28.3	31.7	35
50–64	111	38.7	36.9	22.5
65+	93	37.7	49.5	9.7
Total	371‡	33.2	34.5	27.5

* Number of cases in this column includes 11 "unclassified or other lymphomas."

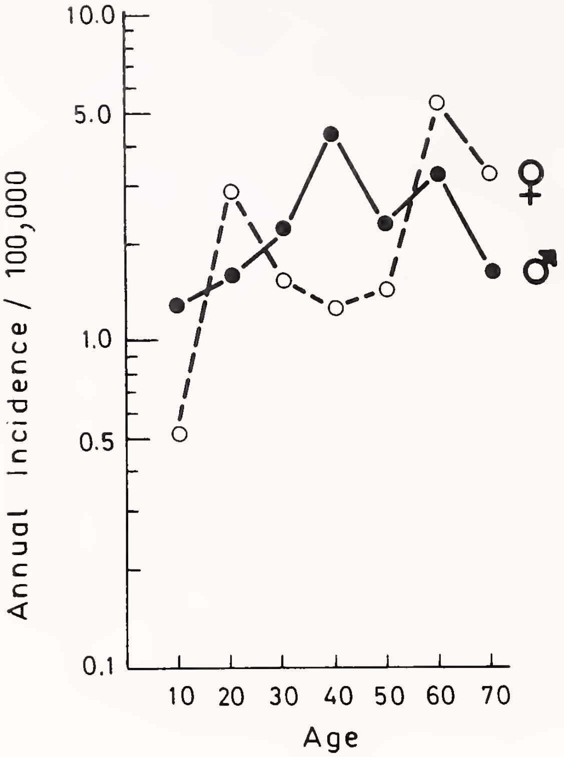
† In this age group, 21.1% of lymphomas were classified as Burkitt-like lymphoma.

‡ Age unknown in 1 case.

Age-Specific Rates for Histologically Confirmed Hodgkin's Disease

The annual age-specific incidence rates for histologically confirmed Hodgkin's disease for the 2-year period 1967–68 are shown in text-figure 2. In the group 5–14 years old, the rates were higher for boys than girls, a finding not evident in the cancer registry figures for the 9-year period, 1960–68. The curve for females showed a clear bimodal pattern, having peaks at ages 15–24 years and 55–64 years, with a distinct trough between these 2 peaks. The bimodal pattern was less clear in males, in whom the highest rate was in the group 35–44 years old. One must, however, remember that the rates for this 2-year period were based on a relatively small number of cases in some of the older age groups.

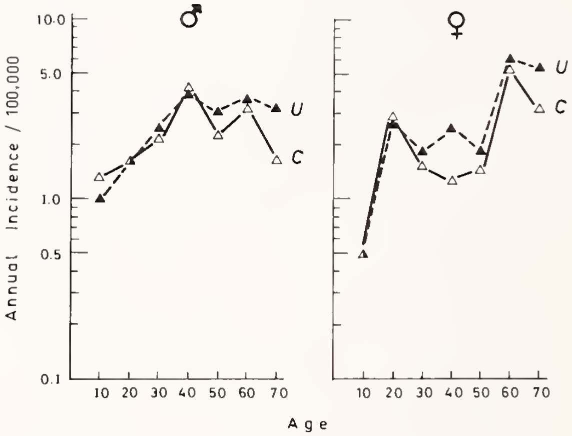
Since cancer registry figures are often used for comparing the rates for Hodgkin's disease in different population groups, it seemed of interest to compare the cancer registry diagnoses with the final diagnosis agreed upon after review of the histologic slides by one team of pathologists. During the 2-year period studied, 117 cases were registered as Hodgkin's disease in the Israel Cancer Registry. In 7 cases, the diagnosis had apparently been based only on clinical or radiologic grounds, and in 3 cases



Text-figure 2.—Age-specific incidence rates for histologically confirmed Hodgkin's disease, by sex. Israel, 1967–68.

histologic sections were unavailable for review. The diagnosis of Hodgkin's disease was confirmed in 91 (85%) of the 107 cases reviewed, whereas 16 cases (15%) were reclassified as reactive hyperplasia (5 cases), lymphosarcoma or reticulum cell sarcoma (9 cases), or nonlymphoid malignancies (2 cases). On the other hand, 11 of 289 cases registered as lymphosarcoma, reticulum cell sarcoma, or malignant lymphoma, type unspecified, during the same 2-year period were reclassified on review as Hodgkin's disease. The reclassified material thus included a total of 102 histologically confirmed cases of Hodgkin's disease as compared to 117 registered cases. The average annual incidence per 100,000 population for histologically confirmed Hodgkin's disease for 1967–68 was 1.96 in males and 1.71 in females. The corresponding rates based on "uncorrected" registry figures were 2.07 for males and 2.15 for females.

The "uncorrected" age-specific rates for Hodgkin's disease for 1967–68 (based on all cancer registry cases) and the "corrected" rates (based on the cases confirmed histologically after review of all malignant lymphomas registered during the 2-year period) are compared in text-figure 3. The curves for the uncorrected and corrected rates showed the same general pattern, the uncorrected rates usually being slightly higher. The differences between the uncorrected and corrected rates in the individual age groups were usually slight (<0.5 or 0.6/100,000), except over the age of 65 in both sexes and in the age group 35–44 years in females. The bimodal nature of the curve in females was more evident for the corrected than for the uncorrected rates. The rates shown in text-figure 3 were, however, based on relatively few cases and it will be interesting to see how corrected and uncorrected rates for Hodgkin's disease compare in a larger series and in cancer registry material from other countries.



Text-figure 3.—Age-specific incidence rates for Hodgkin's disease, by sex. Israel, 1967–68. U = "uncorrected" rates based on cancer registry data. C = "corrected" rates based on cases confirmed histologically after review of all malignant lymphomas registered during the 2-year period.

Hodgkin's Disease in Different Population Groups in Israel

One of the main purposes of the review of all histologically diagnosed malignant lymphomas which we are carrying out in Israel is to obtain more accurate information on the incidence of the various histologic types in the different population groups.

Preliminary results, based on the 2-year period 1967–68, revealed interesting differences between foreign-born Jews according to their continent of birth (*7*). In young adults, 15–34 years old, age-standardized (world population) rates for lymphosarcoma–reticulum cell sarcoma were clearly higher in Jews born in Africa or Asia[4] than in those born in Europe and America. This difference was due, at least in part, to the higher frequency of primary intestinal lymphomas in the Jews of Afro-Asian origin. On the other hand, the rates for Hodgkin's disease in young adults seem to be higher in Jews born in Europe and America than in those born in Africa or Asia. These differences between the 3 main groups of foreign-born Jews with respect to incidence rates for Hodgkin's disease were, however, less evident when cancer registry figures for the 9-year period, 1960–68, were examined. Table 2 shows the age-standardized (world population) rates (*9*) for Hodgkin's disease truncated for ages 15–34 and 35–64 years, on the basis of uncorrected cancer registry figures for 1960–68. (The rates for Israel-born Jews and for non-Jews are not given, since the denominators are so small in some of the older age groups that the rates could be markedly affected by the addition of even a single case.) In both age groups, the rates for Hodgkin's disease were lower in Jews born in Africa than in those born in Europe–America or Asia, particularly in females. The differences between the groups born in Europe–America and Asia were, however, less evident than those found for the histologically reviewed cases for 1967–68 (*7*). We are now extending the histologic review of all registered cases of malignant lymphoma over a longer period to see whether we can confirm the apparent differences between the geographic groups with respect to Hodgkin's disease and, in particular, to see whether any pattern emerges with respect to date of immigration to Israel. It may, for example, be much more important to calculate incidence rates of the disease in the various population groups, among people who were living in Israel, say 10 years previously, rather than to look at rates according to country of birth without taking into account the length of time the patients have lived in Israel. One problem is the widely different age structures of the various population groups in Israel. There is a much lower proportion of children and a much higher proportion of older individuals among foreign-born Jews than among Jews born in Israel or among non-Jews. There are also marked differences in the age distribution of the 3 main groups of foreign-born Jews. Thus, for the years 1967–68, almost 80% of Jews born in Israel and approximately 60% of non-Jews were <20 years old as compared to 8% of Jews born in Europe, 12% of Jews born in Asia, and 30% of Jews born in Africa. On the other hand, 25% of Jews born in Europe–America, 14% of Jews born in Asia, 12% of those born in Africa, but only 5% of non-Jews, and 1% of Jews born in Israel were ≥60 years old.

TABLE 2.—*Age-standardized (world population) rates for Hodgkin's disease per 100,000 population: Foreign-born Jews by sex and age. Israel, 1960–68*

Age (yr)	Males			Females		
	Europe–America	Africa	Asia	Europe–America	Africa	Asia
15–34	2.77 (19)*	2.26 (14)	2.49 (16)	2.40 (17)	1.52 (9)	2.20 (13)
35–64	3.35 (69)	3.10 (13)	3.82 (21)	2.88 (55)	1.84 (7)	2.22 (12)

* Number of cases are given in parentheses. The rates are based on cancer registry data and include both clinically and histologically diagnosed cases.

Histologic Subtypes of Hodgkin's Disease

Sections of the original diagnostic biopsies from 174 cases of Hodgkin's disease were examined by two of us (M.S. and G.S.) and classified according to the Rye modification of the original Lukes-Butler classification (*10*). These included the 102 cases of Hodgkin's disease from the country-wide survey of 1967–68, supplemented by 72 additional cases from those hospitals for which we have completed our review of the sections of all malignant lymphomas diagnosed during 1964–66. While the great majority of cases had been diagnosed as Hodgkin's disease by the pathologist who examined the original biopsies in the various hospitals, the cases of lymphocytic predominance (LP) included 8 cases which had been diagnosed as lymphosarcoma, whereas 4 of the cases of lymphocytic depletion (LD) and 2 of the cases of mixed cellularity (MC) had been diagnosed as reticulum cell sarcoma or lymphosarcoma. This indicates that, in order to obtain a true picture of the relative frequency of the various subtypes of Hodgkin's disease in a retrospective study, the sections of all malignant lymphomas should be reviewed and not merely those cases listed as Hodgkin's disease in cancer registry or hospital records.

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[4] "Jews born in Asia" does not include Jews born in Israel, who are grouped separately.

The age and sex distribution of the 174 patients is shown in table 3. There were 91 males and 83 females. Males predominated in the age groups 5–14 years and 35–44 years, whereas females predominated in the age group 15–24 years.

TABLE 3.—*Distribution of 174 patients with Hodgkin's disease according to age and sex*

Age (yr)	Males	Females	Total
0–4	0	0	0
5–14	14	8	22
15–24	14	24	38
25–34	10	10	20
35–44	16	8	24
45–54	8	8	16
55–64	17	14	31
65–74	8	8	16
75+	4	3	7
Total	91	83	174

In 13 cases, classification was not possible for technical reasons or because only post-treatment or inadequate material was available for study. The frequency of the 4 histologic subtypes in the remaining 161 patients is shown in table 4. Nodular sclerosis (NS) (34%) and MC (30%) were the most common types. LP and LD accounted for 19% and 17% of cases, respectively. LP was more common in males than in females. NS, on the other hand, accounted for 45% of cases of Hodgkin's disease in females but for only 23% in males. The frequency of NS in our material was much lower than that reported in some series from the United States (*11, 12*) but similar to that found by Berard et al. in their Clinical Center study at the National Institutes of Health (*13*). Much lower frequencies of NS were reported from Cali, Colombia (*2*), and Uganda (*14*).

TABLE 4.—*Histologic subtypes of Hodgkin's disease, by sex*

	Number of cases	Percentage in each histologic group			
		LP	MC	LD	NS
Males	83	25	34	18	23
Females	78	13	27	15	45
Both	161	19	30	17	34

The frequency of the histologic subtypes of Hodgkin's disease in the different age groups is shown in table 5. MC was the most common type in children (48% of 21 cases), whereas NS predominated (57% of 54 cases) in young adults, 15–34 years old. In the older age groups, MC was the most common type, but there were not the same marked differences in the fre-

TABLE 5.—*Histologic subtypes of Hodgkin's disease by age*

Age (yr)	Number of cases	Percentage in each histologic group			
		LP	MC	LD	NS
0–14	21	19	48	10	24
15–34	54	15	19	9	57
35–49	29	17	34	27	21
50+	57	25	33	21	21
All ages	161	19	30	17	34

quency of the various types as was seen in children (in whom MC clearly predominated) and young adults (in whom NS clearly predominated).

The high frequency of NS in young adults was seen in both males and females, but this histologic subtype made up a higher percentage of cases of Hodgkin's disease in females than in males in all age groups (table 6). This predilection of NS for females was consistent with that found in series of patients reported from many parts of the world (*11–13, 15–17*).

Table 6.—*Percentage of NS by age and sex*

	Age (yr)			
	0–14	15–34	35–49	50+
Male	15	45	12	16
Female	38	66	33	27
Both	24	57	21	21

The frequency of the histologic subtypes of Hodgkin's disease in the different population groups is shown in table 7. This series included only 13 cases of Hodgkin's disease in Arabs (LP—4 cases; NS—4 cases; MC—3 cases; and LD—2 cases), and they are not listed in the table. The findings shown in table 7 suggest that the various geographic groups differ in the relative frequency of the different subtypes of Hodgkin's disease. Thus, there was a higher proportion of cases of LP among Jews born in Europe–America than among Jews born in Israel or among Jews who had immigrated to Israel from Asia and Africa. The Jews born in Africa and Asia seemed to have a higher proportion of cases of LD than the other groups. These findings must, however, be interpreted with caution until sufficient cases have been examined to permit comparisons between the various geographic groups to be made within individual age groups. The Hodgkin's disease patients born in Europe–America included, for example, a higher proportion of old patients than did those born in Africa. The great majority of the Israel-born patients were children or young adults—the presence of so many young adults in the Israel-born patients explains the higher frequency of NS found in this group.

Table 7.—*Histologic subtypes of Hodgkin's disease by population group*

	Jews born in:		
	Europe–America, 61* (males, 33; females, 28)	Africa–Asia, 44* (males, 22; females, 22)	Israel, 42* (males, 19; females, 23)
LP (%)	24.6	13.6	9.5
NS (%)	32.8	31.8	40.5
MC (%)	26.2	31.8	35.7
LD (%)	16.4	22.8	14.3

* Number of cases.

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Hodgkin's Disease in Childhood [1,2]

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SUMMARY—The incidence, frequency, clinical presentations, and histopathology of childhood Hodgkin's disease in socioeconomically underdeveloped countries differ from those of more affluent societies. In poor countries, the disease is common, chiefly affects boys, and is rarely seen before 4 years of age, after which frequency increases with a severe, aggressive, and rapidly disseminating form with mostly unfavorable prognoses. In developed societies, the opposite situation is seen; with increasing affluence, there are a decline of the disease in children and a shift of the disease impact to a young-adult population. It is a less aggressive disease and has a higher proportion of the more favorable histologic types.—Natl Cancer Inst Monogr 36: 45–47, 1973.

THE VIEW one has of Hodgkin's disease is shaped by the community in which one works. To the medical student in tropical Africa or in some South American countries, Hodgkin's disease is mainly a pediatric problem, with most cases occurring before the age of 20 years; e.g., in the Kenya Cancer Registry, 84 of 145 cases occurred before 20 years of age. In affluent societies, especially in the cities, it is rare in children and is chiefly an adult disease. While the basic features of the disease are the same everywhere (*1, 2*), the incidence, the frequency, the excessive incidence in males, the clinical presentations, and the histopathology vary in different communities and seem to be related to socioeconomic conditions (*3*). In the affluent societies, the disease is relatively uncommon in childhood and the incidence has declined with improved economic and social conditions (*3*). The disease usually presents at an early stage, and there is a concurrence between staging and grading by the Rye subtypes with a preponderance of the Rye types associated with a relatively good prognosis (*4*). But in impoverished societies, the situation is very different.

Among East African children the disease is far more common, with a greater male incidence; it presents in a more aggressive and disseminated form with polylymphadenopathy, organomegaly, frequent extranodal involvement, and severe constitutional symptoms, and the less prognostically favorable Rye subtypes predominate (*2, 4*). Thus, 38.4% of the African children had the lymphocyte-depletion (LD) type as opposed to 9.4% in the Manchester region of England (*4*). The male excess in the African children (boys 140; girls 27) did not account for this discrepancy, since the girls were worse off in this respect (57.4% LD) than the boys. Similar Rye type distributions, or even worse situations, are seen in some South American countries (*3*). On the other hand, in some countries with low socioeconomic levels the disease is uncommon in childhood, e.g., India (*3*).

The aggressive disseminated disease in African children cannot be explained by delay in diag-

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nosis (*2, 5*). The high rates in Africa reflect the large number of affected boys, but the proportion of cases seen in the first quinquennium of childhood is no greater in East Africa than in Manchester (*4*). The disease becomes common in all high-rate countries at about the age of 4–5 years. Tests made in a small group of children who subsequently developed Hodgkin's disease revealed normal immune reactions (*1*), and with the exception of ataxia telangiectasia patients who have developed the disease, no clear association of childhood Hodgkin's disease with congenital defects or anomalies has been observed (*6*). Incidence rates in girls are low in both poor and affluent societies. Correa and O'Conor (*3*) showed that in poor countries there is an incidence peak in childhood, but the early adult peak (*7*) is lacking; whereas with socioeconomic improvement there is a shift in the childhood peak to one of the young adults. We should also note that in more affluent societies the disease has a greater impact on the more economically favored sections of the community.

No other childhood cancer exhibits these peculiarities. The Burkitt lymphoma rarely, if ever, occurs in the first 2 years of life and does not show an excess of cases in males, but the infrequency of lesions of the Waldeyer's ring tissues is similar to that of Hodgkin's disease, in contrast to lymphosarcoma and histiocytic sarcoma. But there is some relationship between Hodgkin's disease and Kaposi's sarcoma which is common in African children and often presents as a polylymphadenopathic disease with a fulminant course. The younger the child, the greater the likelihood that the disease will take this form. In adults, though the lymph glands are involved in many cases, the bulk of the tumorous deposits are extranodal, and the disease rarely takes a fulminant course. There is, moreover, some curious and so far unexplained relationship between Kaposi's sarcoma and Hodgkin's disease which may complicate each other in the same patient. But even with these partial similarities, no other childhood cancer seems to behave epidemiologically, clinically, or pathologically as does Hodgkin's disease.

Miller (*8*) has drawn attention to the change in incidence rates of Hodgkin's disease in the later years of childhood in the United States where a rapid increase in the rates for girls has markedly reduced the male-female ratio. He suggested that this increased incidence rate in the later years of childhood points to the genesis of the childhood disease as a response to some environmental influence or to an abrupt change in susceptibility, and emphasized the possible influence of lymphoid tissue involution that normally takes place in "older children." In our affluent society, surgical ablations of lymphoid tissue are frequently performed; this is not the case in Africa. With data from New York State, we reported an increased liability to develop Hodgkin's disease in persons <40 years old who had had a prior tonsillectomy (*9*). While this can only be a factor in a minority of persons with Hodgkin's disease, it is of interest in connection with Miller's suggestion of the importance of lymphoid tissue involution.

What interpretations can be put on the epidemiologic and behavioral data on Hodgkin's disease in childhood throughout the world are far from clear, and much more information is needed. But all available evidence indicates that the impact of Hodgkin's disease in a society depends on the socioeconomic circumstances of that society. In poor countries, the disease is common, chiefly affects boys, and is rarely seen before 4 years of age, after which frequency increases with a severe, aggressive, and rapidly disseminating form with mostly unfavorable prognoses. In affluent societies, the opposite situation is seen; with increasing affluence, there is a decline of the disease in childhood and a shift of the disease impact to a young-adult population. It is a less aggressive disease and has a higher proportion of the more favorable histologic types. In almost all communities, the disease affects young females less often.

By analogy with other diseases, it is easy to see in these features resemblances to an infectious disease rather than to other cancers common in childhood and adolescence. With socioeconomic improvement, the age impact of several known infectious diseases shifts from young children to older persons. The infrequency of the disease in very early childhood in all societies suggests a long incubation period, and this is in agreement with other evidence that we have and the observation that the spread of Hodgkin's disease resembles a slowly contagious infective disease spread by case to case or by case to contact to case (*10*). Indeed Fraumeni and Li (*6*) suggested that exposure to some environmental agent around the time of birth might be important in the causation of childhood Hodgkin's disease.

Further studies of childhood Hodgkin's disease are needed. If an infectious agent is involved, the chances of isolating it in the acute, aggressive form of the disease in African and South American children would be better than in those children and adults in affluent countries with what appear to be more resistant types of the disease. Such studies can now be undertaken in more favorable circumstances since, at least in the severe, aggressive types of Hodgkin's disease of children in Uganda, the response to chemotherapy alone is remarkable, with a 96% remission rate (personal communication); there, where I have unhappy memories of autopsying many children with Hodgkin's disease, no treated child has died of this disease in the last 2 years.

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SUMMARY OF INFORMAL DISCUSSION ON GEOGRAPHIC PATHOLOGY OF HODGKIN'S DISEASE

G. T. O'Conor underscored the point made by Professor Davies that in regions exhibiting pattern I, with its high incidence in children, the male predominance is also very striking. Another point he emphasized was table 4 of Professor Correa's presentation and the figures from Tunisia and Peru, where 67% of the cases are of the lymphocyte-depletion type. This most unusual observation indicates that these are places where one ought to look for some environmental factor that plays a very prominent role.

H. S. Kaplan asked whether the very high incidence from the coastal areas of Peru, as well as the data that Professor Wright presented, indicating a lack of correlation with the distribution of malaria within Uganda, would indicate that we already have sufficient data to conclude that malaria is not a cofactor. Secondly, he asked what is known about the nutritional patterns in the areas in which these remarkably high frequencies of the more aggressive type of disease are seen, particularly in children. For example, what is known about consumption of milk or other dairy products? He had had the preliminary impression that pattern III, with a low incidence in children and a high incidence in young adults, is seen in countries in which there is a high consumption of dairy products. He wondered whether the countries that do not show this may have a lower consumption of dairy products.

P. Correa replied that the incidence of malnutrition is very high in all the subtropical countries of Latin America, and that in Peru the population has the highest rate for tuberculosis of all Latin Americans. This usually goes hand in hand with malnutrition in children in these countries. He did not have the data on malaria. Malaria occurs in Latin America, but it is nowhere near the incidence or severity that is seen in Africa. **R. A. Good** commented that the effect of malnutrition on immune capacity has been receiving considerable attention of late, and is certainly a topic that one would like to concentrate on in the study of Hodgkin's disease.

D. Wright stated that a number of studies have been carried out in East Africa on cutaneous delayed-hypersensitivity reactions and lymphocyte reactivity among normal children and children with various diseases. These studies reveal no evidence of any measurable decrease in cell-mediated immunity. He pointed out that malaria is a disease that strikes everybody in certain parts of Uganda. One gets it repeatedly until he is either killed by it or gets immunity. This means that a certain type of person is selected out, and the population remaining is abnormal. Knowing what we know about cellular immunity deficiencies in patients with Hodgkin's disease, such a disease apparently could affect the distribution of Hodgkin's disease. Professor Wright had looked very carefully at the Uganda figures from this point of view and analyzed them by the sorts of parameters that were available; i.e., he looked at incidence in each area for Hodgkin's disease as compared with total cancer incidence and found no evidence of geographic variation.

Good commented that studies of the relationship between nutritional deprivation and immunity are extremely difficult to design. The complicated interactions between pestilence and nutritional deprivation do not suit themselves to sorting out in population studies. Laboratory investigations are necessary to bring these things into focus. In experimental work, highly controlled studies clearly indicate that modern protein deprivation does not affect cellular immunity. As a matter of fact, it may enhance cellular immunity while at the same time depress antibody production. More profound malnutrition will affect both cellular and tumor immunity. Thus, we have to be very careful not to draw conclusions about the influences of nutrition on immunologic processes until we can quantitate precisely both the nutritional deprivation and the separable immunologic functions and can analyze the influence of definable microbial flora on the immunologic response.

G. Newell commented that the original observation that Doctor McMahon made is still very pertinent. The central issue is that age is a definite risk factor in the development of Hodgkin's disease. How one interprets it is relatively immaterial at this time. We are now beginning to relate age to other factors, such as histologic type.

M. Tubiana remarked that, in the European cooperative clinical trial, all the slides were reviewed by a committee of pathologists. Even though the initial diagnoses were made by experienced pathologists, the discrepancy between the initial diagnosis and the final diagnosis was about 5% and, furthermore, the difference in histologic subtyping was about 25%. Tubiana believed that such discrepancies should be kept in mind in comparing histologic subtyping from different institutions and countries. He added that in France the age distribution of the various histologic subtypes is very different in males and females. Therefore, age distribution data of the subtypes which do not present a breakdown between male and female are extremely difficult to interpret. Thirdly, there is a very strong correlation between clinical stage and certain histologic subtypes. The more aggressive histologic subtypes are associated with advanced stages. We must keep this in mind when comparing data from different countries. If in a given country the diagnosis is usually made at an advanced stage, it may be expected to reveal a high proportion of cases of adverse histologic subtype.

Part 2. Virology and Serology

Chairman: M. A. Epstein

INTRODUCTION

As an introduction, it might be worth pointing out that there has been a considerable revolution in our thinking on viruses and cancer. Even as recently as 10 years ago, it would have been absurd to consider that anybody except mice or chicken could have had virus-induced tumors. The idea that viruses could play a role in human neoplasia would have been deemed very bizarre. Yet here we are, 10 years later, with a session in an important Symposium like this devoted to "Virology and Serology," and prepared to consider the very serious possibility that viruses are involved in the etiology of Hodgkin's disease in man. We will open the proceedings with Dr. David A. Stevens, of the Department of Medicine at Stanford University Medical School, who is going to give us a survey of the oncogenic herpesviruses and their possible relationship to Hodgkin's disease.

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Oncogenic Herpesviruses: A Selective Review and Their Possible Implications for Hodgkin's Disease [1,2]

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SUMMARY—The herpesviruses are phylogenetically widely distributed in nature, and can cause persistent latent infection. Herpesviruses have been shown to cause lymphoproliferative diseases, including lymphoma and leukemia, and other tumors in several animal species. Two herpesviruses of man have been implicated in several human tumors. Epidemiologic and histologic observations have suggested that Hodgkin's disease may be caused by an infectious agent. Our knowledge of the herpesviruses is reviewed because of the possible role of these viruses in this disease.—Natl Cancer Inst Monogr 36: 55–63, 1973.

THE HERPESVIRUSES are a group of DNA viruses icosahedral in shape, possessing 162 capsomeres, and sensitive to ether. They are phylogenetically widely distributed in nature and are clearly the cause of neurologic, respiratory, genital, ocular, and other diseases in their natural hosts in several species (*1, 2*). More recently, attention has been drawn to these agents as a cause of cancer in several species. Dr. H. S. Kaplan has charged me with the task of giving an overview of this area and juxtaposed this with the question of the possible relationship of these agents to Hodgkin's disease.

Why *should* we consider these viruses in relation to Hodgkin's disease? The foremost reason is the suggestion that Hodgkin's disease is caused by an infectious agent. Provocative epidemiologic evidence for this hypothesis has been reviewed at this Symposium. Perhaps of particular note are the similarity of Hodgkin's disease histologically to an inflammatory response that has intrigued pathologists for decades and the reports of seasonal variation, clustering, intrafamilial spread, and the enhancing effect of prior appendectomy and prior tonsillectomy on the incidence of the disease (*3*). The herpesviruses infect early in life, are capable of persistent latent infection, and cause disease at a greatly increased rate when immune defenses are lowered (*4*). Therefore, they might be prime suspects for involvement in neoplastic transformation as well. The facts that they do cause tumors in other species, both naturally and under laboratory conditions, that certain members are lymphotropic, and that some of the induced tumors involve the lymphoid system are the greatest reasons for their being scrutinized. Since we will never be able to fulfill Koch's postulates with any candidate human tumor virus, and since cancer virology is likely to be less straightforward in our "noninbred" species, man, we must learn all we can about these oncogenetic mechanisms for potential analogies. Most exciting of all is the possible role of a human herpesvirus, discovered by Epstein et al. (*5*), in causing human tumors (*6–8*). At the very epicenter of the problem is evidence that this same agent might be involved in Hodg-

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported in part by Public Health Service grant AI05629 from the National Institute of Allergy and Infectious Diseases.

kin's disease itself. This will be the subject of the talk by W. Henle[3] to be presented at this Symposium.

The herpesvirus-tumor system most phylogenetically distant from man is the renal adenocarcinoma of frogs (*9–13*) (table 1). The incidence of these tumors in nature in some frog strains seems uniquely high for spontaneous tumors in wild animals. Oncogenesis appears to be influenced by age and hormonal and genetic considerations, since the risk is higher in males and with advancing age, and not all breeds are susceptible. Documentation of a viral etiology was delayed by the high incidence of spontaneous tumors at room temperature in the laboratory, so that the incidence with injected materials was masked. However, recent reports of cell-free transmission with zonal-centrifuge fractions of high virus content appear to clinch a herpesvirus as the etiologic agent. In vitro, thus far, only a lytic effect of the virus has been demonstrated. The virus is most likely transmitted horizontally. Winter tumors, or tumors in frogs under low temperature in the laboratory, produce virus and have typical herpetic inclusion bodies. Explants will show typical herpes cytopathic effect (CPE), in contrast to summer tumors, or tumors in frogs kept at room temperature. Frogs emerging from winter hibernation also have viruria, and contamination of the water in which the tadpoles grow is a likely vehicle.

TABLE 1.—*Herpesvirus-tumor systems*

Species	Tumor system
Frog	Renal carcinoma
Chicken	Lymphoma (Marek's disease)
Rabbit	Lymphoma
Simians	Lymphoma and leukemia
Man	Herpes simplex: carcinoma of cervix? Epstein-Barr virus: Burkitt's lymphoma?, Hodgkin's disease?, nasopharyngeal carcinoma?, and chronic lymphocytic leukemia?

A loss of an organ-specific antigen from the tumor has been shown, as well as the presence of an antigen in the tumor and in tadpole cells but not in normal kidney. This last situation is reminiscent of the carcinoembryonic antigens in man described in relation to colon carcinoma. The observation that metastases are rare in the field but more common in the laboratory is poorly understood.

The most dramatic advances with any system are those that have been made in Marek's disease or neurolymphomatosis of chickens, a disease, first described in 1907, of great economic importance to the poultry industry (*14–32*). The disease is neurotropic and paralyzes, and lymphoid tumors are prominent also in the ovary and skin. Two forms have been described: 1) acute, with high mortality and rapid progression, and 2) the "classical" or more indolent form. The existence of these 2 forms appears to be related to virus strain differences. Other biologic or immunologic markers which could distinguish these virus strains have not been described. Work was impeded because it was difficult to pass an agent into tissue culture, a feat achieved by 2 groups, working independently, 60 years after the discovery of the disease. On reinoculation, the tissue culture isolates could reproduce the disease and were shown to be herpesviruses. Most striking was the intimate association of infectivity with intact cells in vivo and in vitro, in contrast to the ease of natural transmission. (Heterosexual transplantation had shown, by the sex chromosome marker, that the transmitted disease was true induction and not a transplantation.) This mystery was unraveled when it was shown that enveloped virus is produced in feather follicle epithelium, from which cell-free infectivity can be demonstrated in vivo and in vitro (as has now also been achieved with tissue culture material). This also helps explain the earlier demonstrations of the infectiousness of dander and dust from the litter of infected birds, of airborne transmission, and of the infectiousness of the environment of affected birds for several months. It has been further documented experimentally that dried feathers alone, without other contamination from affected birds, are highly infectious.

Several facets of the biology of this system are of potential interest to students of human disease. Young birds and females, in contrast to the frog system, are most susceptible. Viremia and virus shedding occur before the disease appears. Appearance of the disease is marked by atypical cells in the blood, hyperglobulinemia of the 7S type, a drop in body temperature followed by fever, and a positive serologic test (Kahn test) for syphilis ("biological false positive"?). Oral and nasal washings are infectious, and, with some virus strains, also feces. In some animals that survive, viremia and shedding continue. The high prevalence of precipitating antibody in adults and the observations that sometimes chickens could be protected by exposure to the litter of older birds suggest the existence of virus strains of low pathogenicity and the occurrence of inapparent infections. This may have relevance to observations with precipitating antibody against Epstein-Barr virus (EBV) in humans. Insects may participate in horizontal spread, though it is not known whether they function as carriers or intermediate hosts. Whether vertical transmission occurs is currently being debated. It seems neither of these 2 methods of transmission is necessary or important under natural conditions.

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Genetic differences in susceptibility appear to be mediated by resistance to development of disease or control of internal virus spread, rather than resistance to infection. Antibodies can be shown in resistant strains exposed to a virulent strain of virus. Different chicken strains also show different preferential sites for involvement. Experiments on selection for resistance suggest it is a recessive trait under control of a single gene or a few genes. In some experiments, a mutant hemoglobin type has been associated with resistance. There appears no cross-resistance with the induction of tumors by Rous sarcoma virus.

The immunology of this system is also very interesting. Impairment of antibody production in infected birds begins during the incubation period, and defective graft rejection occurs also after the disease has become apparent. Passive transfer of antibody can be protective, but passive antibody transfer through the egg does not overcome the enhanced susceptibility of young birds. Young chicks may be infected and die of the acute form without the appearance of endogenous antibody. This suggests difficulties if human tumor populations are studied solely by serologic means to identify an etiologic agent. Incompletely understood at present are the mechanisms underlying spontaneous regression in less severe cases and the effect of bursectomy in decreasing mortality.

The virus behaves differently in different cells in tissue culture. In chick embryo cells, a rounded type of CPE is seen, and enveloped particles and cell-free infectivity can be easily demonstrated. In duck embryo cells, by contrast, a syncytial type of CPE is seen. These differences in behavior appear specific to the cell and unrelated to the passage history of the virus.

Of particular note in regard to a possible human parallel is that the tumor itself cannot be shown to contain the virus by electron microscopic examination or by immunofluorescent testing for viral antigens, but can be shown to contain the genome by cocultivation with susceptible cells or by demonstration of cell-associated infectivity. The bursa of infected birds has immunofluorescence-reacting viral antigens but not the complete virus particle, and the cells are infectious but cell-free infectivity cannot be shown. Curiously, although immunofluorescence-positive, bursal cells appear to generate less cell-associated focus-forming activity than tumor cells. Complete replication of naked virus particles has been demonstrated in kidney tubule epithelium of infected birds, and the enveloped, fully infectious variety occurs in the feather follicle epithelium. The possible role of the cooler body temperature at this site in this phenomenon is an intriguing parallel with the temperature dependence described in the frog system.

Most exciting of all are the prospects for control of the disease. Initial efforts were directed to genetic selection, but of more promise to man was the development of effective vaccines. The wild virus has been attenuated by passage in tissue culture (initially in chick kidney cells and now also in chick embryo and duck embryo cells), and infection with this agent protects against the disease. However, infection with the wild virus occurs, and both the attenuated and the virulent viruses persist in the host. It is of interest that the attenuated virus appears on immunodiffusion analysis to have lost one antigen; otherwise all the isolates appear identical in gel diffusion. No reversion to virulence of the attenuated virus has been noted. Infection with an antigenically similar herpesvirus of turkeys has also been shown to protect against disease, but again, not against infection with the wild virus. Like the attenuated viruses, the turkey herpesvirus is poorly contagious, though it causes a persisting viremia in the host. Since cell-free virus can be produced easily, it offers potential advantages for vaccine development. Finally, a naturally avirulent wild-type virus has been studied and used as a vaccine.

The mechanism of resistance after vaccination, i.e., the relative contributions of humoral immunity, cellular immunity, viral interference, or interferon, has not been delineated.

The observation that in vivo assay is more sensitive than any in vitro assay may be discouraging to workers who attempt isolation of agents from human tumors.

A herpesvirus causes a lymphoma in young wild cottontail rabbits (*33*). The incubation period, 6–8 weeks, is surprisingly short for usual viral oncogenesis systems, and thus parallels the system to be described next. Also of note is that, in mature animals, a benign lymphoproliferative disease can be produced, resembling infectious mononucleosis.

With *Herpesvirus saimiri,* a virus indigenous to the squirrel monkey, we find an oncogenic herpesvirus in our own phylogenetic order, the primates (*34–36*). This virus can produce lymphomas, leukemia, or a benign lymphoproliferative disorder in owl monkeys and marmosets. (This seems a variation on the older observation that some primate herpesviruses can be usually benign in their indigenous hosts but usually lethal when they cross over into another primate, e.g., herpes B virus of rhesus monkeys into man, but such lethality has been associated with lytic infections.) This virus can be reisolated from the blood, marrow, visceral organs, and tumors of affected animals. Moreover, from the tumors, lymphoblastoid suspension cultures replicating the virus have been obtained, a situation reminiscent of Burkitt's lymphoma and EBV. The virus can infect human cells but, in these and simian cells in vitro, has thus far only been shown to have a lytic cycle.

Herpes simplex, the common "cold-sore" virus, has been shown to enhance 3-methylcholanthrene oncogenesis in rodent skin (*37*). It can transform hamster embryo cells in vitro; these cells cause tumors on injection into hamsters (*38*). It has been speculated that it causes squamous cell carcinoma in lip sites of herpetic recurrences. Much more consideration has been given to the possible etiologic role of herpes simplex in carcinoma of the cervix (*39–43*). Epidemiologic data associating this tumor by several parameters with sexual activity suggest a venereal etiology. A biologically and immunologically distinct strain of herpes simplex, type 2, frequently results in apparent and inapparent (cytologically detected) infections of the female genitalia, particularly in females during the years of sexual activity (*44, 45*). Prevalence of antibody to this virus increases during the sexually active years, and antibody and infections are more common in more sexually active groups. In some surveys, it has been the only virus agent recovered from cultures of male genitalia and smegma. Several centers have reported an increased incidence of type-2-specific antibody in women with cervical carcinoma compared to control populations (often loosely matched), but there is not complete agreement on this point. A latent type-2 virus has been uncovered by in vitro manipulations of a continuous cell line derived from a cervical carcinoma. If other venereally transmitted infections are not associated more frequently with carcinoma of the cervix, as the better matched comparisons suggest, the increased antibody may indicate significance beyond covariability of cervical carcinoma and genital herpes in association with increased sexual activity. An increased incidence of this antibody in patients with cervical dysplasia and in carcinoma in situ of the cervix has been reported, and a >20-fold increase in the incidence of carcinoma in situ has been reported in patients with genital herpes. A significantly greater incidence of antibodies against type-2 virus was found in a group of cytologically normal patients who subsequently developed the disease. The peak age of genital herpes in the population precedes by many years the peak age for carcinoma in situ, and a similar relationship exists for carcinoma in situ and invasive carcinoma. This is suggestive epidemiologic evidence for an etiologically linked progression between these conditions. However, the increased incidence in some series of antibody in patients with invasive carcinoma compared to carcinoma in situ, despite the similar epidemiology of these 2 diseases and the premise that carcinoma in situ is an early stage of invasive carcinoma, speaks more for an increased susceptibility of carcinoma cells or patients with carcinoma to infection with the virus, or possibly a specific etiologic role in frank carcinoma not present in carcinoma in situ. In addition to the biologic markers distinguishing type-1 and type-2 viruses, neutralization studies suggest the genital virus strains (type 2) lack antigenic sites present in nongenital strains (type 1).

More relevant to students of Hodgkin's disease are the findings reported from several laboratories, with several immunologic techniques, of increased antibody to EBV in the 4 human tumors shown in table 1. The story begins in Africa with the observations by Denis Burkitt of a childhood tumor of unusual frequency (responsible for a 3- to 25-fold increase of childhood lymphoma in the areas where it is common). This tumor has a predilection to involve the jaw, ovary, and nervous system. It is of rather uniform (though not pathognomonic) histologic appearance (histiocytes sprinkled among primitive lymphoreticular cells, giving a "starry-sky" appearance) (*46–48*). Epidemiologic investigation in Africa showed a unique association of this tumor geographically with temperature and rainfall conditions which suggests an insect-vectored etiology. Moreover, areas with appropriate climatic conditions but with mosquito eradication programs (e.g., Kinhasa) appear to be "tumor-free." Further evidence supporting an infectious etiology is the finding of time-space clustering and a peak age incidence of 3–5 years in the endemic areas, with cases appearing in older individuals in areas with fewer cases. This suggests a common infection in early childhood, with a short incubation period for oncogenesis in a few individuals and immunity in the remainder. In low incidence areas, some individuals would escape infection in early childhood, and older, nonimmune individuals would be susceptible to infection and occasional oncogenesis. In accord with this formulation, people from low incidence areas (such as those without appropriate climatic conditions) only develop the tumor when they migrate to high incidence areas to work, and in the latter areas these "susceptibles" are responsible for the cases occurring outside the early childhood group. Evidence of host response to the tumor—apparent clinical cures (often with low dose chemotherapy), reports of spontaneous remission, serologic evidence of a response to antigens in autologous and allogeneic tumors, and cutaneous delayed hypersensitivity to autologous tumors—suggests the tumors stimulate an immune response because of new antigens coded by an infectious agent. There may be a difference in susceptibility for patients with the S hemoglobin mutant.

As these epidemologic data were being gathered, in vitro cultivation of tumor explants was achieved in several laboratories. Electron microscopic examination by Epstein of cultures growing in his laboratory revealed the presence of a hitherto undiscovered human herpesvirus (*5*). At this time, long-term tissue cultures of the peripheral blood or lymph nodes of patients with leukemia and other disorders and of normal individuals were achieved at Roswell Park Memorial Institute and elsewhere (*49*). These cultures and the Burkitt tumor cultures yielded cells of similar lymphoblastic morphology which grew rapidly in suspension and appeared immortal. Most of these cultures on examination contained a morphologically similar herpesvirus (*6–8*). By analogy with the preceding systems, cultivation at cooler temperatures enhanced viral replication.

Seroepidemiologic studies, first performed by the Henles by testing antibody by immunofluorescence on culture cells and then by other immunologic techniques in other laboratories, have shown that infection with this virus is nearly universal by adulthood but that titers are uniquely high in Burkitt's lymphoma patients and also elevated in other neoplastic diseases (table 1). Table 2 shows this information as derived from immunodiffusion assay in our laboratory (*50*). By this method, 92% of Burkitt lymphoma patients are seropositive as compared to 60% of American adults. Further excitement about the virus was generated by serologic evidence (*51, 52*), from retrospective and prospective studies, that it is the agent responsible for infectious mononucleosis. The relationship with this benign lymphoproliferative disorder is particularly intriguing because of histologic similarities with Hodgkin's disease (e.g., Reed-Sternberg cells) and other lymphomas, the presence of heterophil antibody in lymphomas, and reports of Hodgkin's disease developing after infectious mononucleosis (*53*). The statistical significance of the last phenomenon is presently under study. Furthermore, a lymphoproliferative effect of this virus has now been demonstrated in vitro by several groups, and Dr. Gerber will tell you about the very exciting developments in his laboratory (*54*).

TABLE 2.—*Summary of immunodiffusion data**

	Mean No. of bands	Percent positive	Percent ≥ 2 precipitin bands	Percent ≥ 3 precipitin bands
I. Pediatric groups				
1a. Burkitt lymphoma sera (119)†	1.74	92	51	25
1b. Burkitt lymphoma patients (73)‡	1.73	92	50	25
2. American healthy individuals (120)	0.33	31	3	0
3. Acute lymphocytic leukemia (14)	0.50	43	7	0
II. Adult groups				
1. American healthy individuals (141)	0.79	60	18	2
2. Acute myelocytic lekuemia (17)	0.71	65	6	0
3a. Infectious mononucleosis sera (42)	0.84	64	16	2
3b. Infectious mononucleosis patients (28) ‡	0.75	61	11	4
III. Inclusive				
1. African healthy and "other pathology" (30)	0.87	63	16	3
2. American controls (261)	0.58	46	11	1
3. All acute leukemia (31)	0.61	55	6	0

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† Total sera tested in parentheses.

‡ Serial sera were available from some patients, and the data are also summarized using only the first sample available from each patient.

I would like to close by considering 3 critical questions presently unanswered. First, is the virus etiologic in the human tumors mentioned? An exciting prospective study in Africa in a Burkitt's-tumor endemic area by the International Agency for Research on Cancer is under way and may give us a critical answer regarding the serologic status in the population at risk (*55*). Studies performed in collaboration with P. H. Levine (*56*) on chronic lymphocytic leukemia, indicating that the high titers are present early in this disease and precede and are unrelated to the marked lymphocytosis, suggest to us that the high antibody reactivity is an early event and does not merely reflect replication of a passenger in a large tumor. Analogous data have been presented earlier for Burkitt's lymphoma. Second, is Burkitt's lymphoma one entity or are the clinicopathologically identical cases, seen outside Africa, etiologically unrelated? Our study (*57*) of a series of American patients with this entity did not show an increased hyperreactivity to EBV compared to controls, as is seen in Africa. Further complexities in this situation appeared to us (*58*) when we discovered recently the simultaneous onset in 2 siblings in California of classical Burkitt's lymphoma in one sibling and an acute leukemia with cells cytologically and histochemically identical to Burkitt's lymphoma cells, a new clinical entity, in the other sibling. The simultaneous onset in these cases suggests a short incubation period. One is easily reminded that the simian oncogenic herpesvirus can cause lymphoma or leukemia with a short incubation period. Third, are the EBV's associated with these different conditions and with normal people the same or different? We studied (*59*) this problem with immunodiffusion analysis (table 3) and found immunologic identity of viruses derived from different tumors. On the other hand, a morphologically identical lymphocyte-culture-associated chimpanzee virus was antigenically different (*60*). Possibly this has parallels with the various Marek's disease isolates on the one hand and the attenuated strain and the turkey herpesvirus on the other. Most important is whether these viruses are biologically different or what cofactors may be involved if they cause neoplastic disease. It is of interest that malaria infection of mice enhances the development of virus-induced lymphomas (*61*).

TABLE 3.—*Immunologic identity of viruses from different tumors**,†

Cell line or tissue source	Source of cell line	Previous history of EBV	Percent cells fluorescing before aging	Percent cells fluorescing after aging	Demonstration of precipitin bands identical with reference sera	Cell line reference
I. Cell lines						
1. P3J	BL, Africa, tumor	+	8–10	10–15	A, B, C	Pulvertaft, 1965
2. HR1K	BL, Africa, tumor	+	18.5–22.5	25–30	A, B, C	Hinuma et al., 1967
3. F152	ALL, brain	+	5–10	10–14	A, B, C	Mayyasi et al., 1967
4. F132	RCS, node	+	10	10–15	A, B, C	Jensen et al., 1967
5. EB3	BL, Africa, tumor	+	5.5–6.5	6–7	A	Epstein et al., 1965
6. SH1P	BL, USA, blood	+	<0.5	<0.5	0	D. A. Stevens, unpublished
7. NC37	Normal, blood	—	0	0	0	E. M. Jensen, personal communication
8. Raji	BL, Africa, tumor	+ and —	0.5	1.5	0	Pulvertaft, 1965
9. RPMI 1348	MM, blood	—	<0.02	<0.02	0	Moore et al., 1967
10. RPMI 3306	CML, blood	+	0	0	0	Moore et al., 1966
11. PGLC 55E	IM, blood	+	<0.2	<0.2	0	Moses et al., 1968
12. F126	Lung CA, node	+	2–3	2–4	0	Jensen et al., 1967
13. NHDL-5	Normal, blood	+	<0.5	<0.5	0	Gerber and Monroe, 1968
14. RPMI 6410	AML, blood	+	<0.5	<0.5	0	Iwakata and Grace, 1964
II. Tissues						
1. BL, USA, tumor			0		NT	
2. BL, USA, tumor‡			0		0	
3. BL, USA, blood§			0		NT	
4. BL, USA, tumor			NT		0	
5. CML, buffy coat			0		0	
6. CML, spleen			NT		0	
7. ALL, spleen			NT		0	
8. CLL, node			NT		0	
9. RCS, node			NT		0	
10. Hodgkin's disease, node			NT		0	

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† *Abbreviations*: BL—Burkitt's lymphoma, ALL—acute lymphocytic leukemia, RCS—reticulum cell sarcoma, MM—malignant melanoma, CML—chronic myelocytic leukemia, IM—infectious mononucleosis, CA—carcinoma, AML—acute myelocytic leukemia, CLL—chronic lymphocytic leukemia, NT—not tested, and 0—no reaction.

‡ From same patient from whom the SH1P cell line was derived.

§ Same specimen placed in culture resulted in SH1P cell line.

ADDENDUM

Presentation of a comprehensive bibliography in this vast area was not possible, and maximum use of recent reviews has been made in the references. The author apologizes to those scientists whose work has advanced this field and whose results I have quoted without adequate citation.

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Is Epstein-Barr Virus a Human Tumor Virus?[1]

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SUMMARY—Transformation of human lymphocytes by Epstein-Barr virus (EBV) is characterized by changes associated with malignant transformation of mammalian cells by oncogenic DNA viruses. EBV activates cellular DNA synthesis and the virus-infected cells appear to be released from the normal regulatory control mechanism. This is indicated by the capacity of EBV-transformed lymphocytes to grow autonomously in culture and to invade, metastasize, and kill immunosuppressed mice on heterotransplantation. Although there is at present no direct evidence supporting a causal relationship of EBV to human neoplasia, the unique biologic properties of EBV suggest a role other than that of a mere passenger.—Natl Cancer Inst Monogr 36: 65–71, 1973.

SINCE THE viral etiology of avian, murine, and feline leukemia and sarcoma was firmly established, a concentrated effort to detect and isolate human oncogenic RNA viruses has been under way. While the tempo of this activity has increased noticeably in recent months, resulting in the nomination of several candidates, interest in the search for an oncogenic DNA virus in man has somewhat diminished. The reason for this may lie in the discovery that the oncogenic properties of certain human adenoviruses were expressed only in experimental animals. The firmly established etiologic relationship of a herpes-type virus to Marek's disease in chickens (*1*) and the discovery by Epstein and Barr (*2*) of a unique and distinct herpes-type virus in cultured cells from Burkitt's lymphomas revived interest in the possible role of DNA viruses, particularly herpesviruses in human neoplasia.

Serologic and clinical data provided good evidence for the causal relationship of Epstein-Barr virus (EBV) with infectious mononucleosis (*3*). The case for the possible association of EBV with Burkitt's lymphoma and nasopharyngeal carcinoma rests mainly on the observation that patients with these diseases have significantly higher EBV antibody titers than matched controls from the same region (*4*). Perhaps more direct evidence for the intimate association of EBV with Burkitt's lymphoma and nasopharyngeal carcinoma was provided by the detection of viral DNA in tumor biopsies (*5*). This finding, however, does not eliminate the possibility that the tumor cells became secondarily infected with this virus, which does occur in lymphoid cell cultures derived from healthy persons (*6*).

The question raised in the title of this paper can be divided into two parts: 1) Is EBV oncogenic in man? 2) Does EBV possess biologic properties similar to those associated only with known oncogenic DNA viruses? Since available evidence is insufficient to provide meaningful answers to the first question, I shall limit this discussion to present some evidence indicating that EBV indeed shares several important properties with oncogenic DNA viruses.

I shall briefly review some characteristics of EBV. The ultrastructural features of EBV are typical of those of the herpesvirus group. Immature particles, present in both nucleus and cytoplasm of infected cells, measure 75–80 mμ in diameter; the mature enveloped particles, found only in the cytoplasm, have a diameter of 110–120 mμ. The virus nucleoid consists of DNA with a guanine-cytosine content of 58–60 moles percent. The viral capsid has an icosahedral symmetry and appears to consist of 162 capsomeres. EBV is not restricted to Burkitt's lymphoma cells but has been found in lymphoid cell lines derived from both malignant and nonmalignant sources. It is generally detectable in relatively few cells (0.5–3.0%); some cell lines appear to be "virus free." Available evidence indicates that EBV infects only cells of the human lymphoreticular system. EBV is serologically distinct, and isolates obtained from various sources seem to be antigenically identical. This finding, however, does not exclude the possible existence of biologic strain differences. EBV is disseminated throughout the world population, with an age distribution similar to herpes simplex virus. It is of interest that old-world subhuman primates are infected with a closely related virus in their natural habitat (7).

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

As a basis for comparison let us now briefly examine the characteristic properties of oncogenic DNA viruses and the cells transformed by these agents. These viruses can infect certain "permissive" cells causing a productive, lytic infection, whereas infection of "nonpermissive" cells is nonproductive and abortive and may result in morphologic transformation. Some of the characteristic features of this system are listed below.

Characteristics of Oncogenic DNA Viruses

1) Induction of cellular DNA synthesis
2) Morphologic transformation of certain cells
   a) Loss of contact inhibition
   b) Unlimited growth in vitro
   c) Invasive tumors in suitable animals
3) Transformed cells usually free of infectious virus
4) Viral genome present and inducible by cell fusion or mitomycin C—simian virus 40 (SV40)
5) Virus-specific neoantigens and transplantation antigens

Infection with oncogenic DNA viruses induces host DNA synthesis followed by morphologic transformation. As a result of loss of contact inhibition, the transformants tend to form multilayered foci, and they attain unlimited growth potential in vitro. When inoculated into syngeneic animals, the transformed cells produce invasive tumors. Infectious virus is usually absent in transformed cells, but viral genetic material has been demonstrated by genome rescue, chemical induction, or nucleic acid hybridization. Indeed, the maintenance of the transformed state appears to depend on the continued presence of at least a portion of the viral genome. Finally, cells transformed by oncogenic DNA viruses contain intracellular, virus-induced, neoantigens or T-antigens and new surface antigens.

With this background in mind, I shall now review the current state of knowledge regarding the biologic properties of EBV. During recent years, it has become increasingly evident that the success of establishing long-term leukocyte

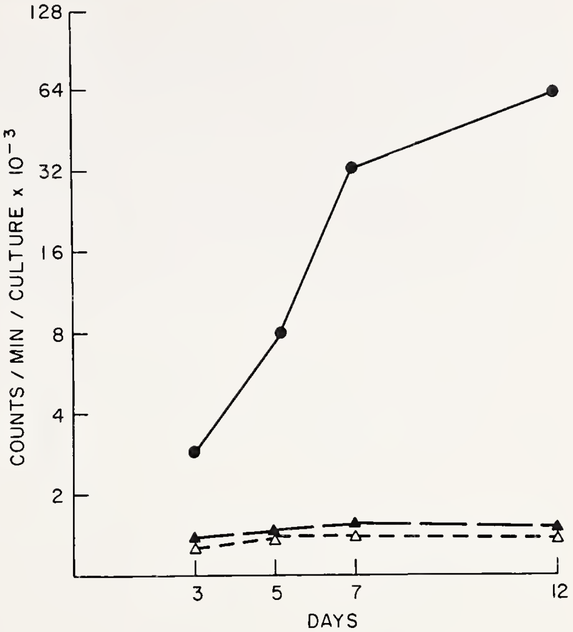
Table 1.—*Leukocyte cultures from healthy donors with and without antibodies to EBV*

Leukocyte culture No.	Days of survival or growth	Autonomous growth after days	EBV by immuno-fluorescence	Complement-fixing antibodies of donor to EBV
1	Infinite	30	+	16
2	Infinite	38	+	8
3	Infinite	60	—	32
4	Infinite	41	—	128
5	Infinite	48	+	64
6	Infinite	52	+	64
7	Infinite	59	+	4
8–25	38–96	—	—	<2
26–40 cord	21–120	—	—	Maternal

cultures depends on prior infection of the donor with EBV. Table 1 lists representative examples from our laboratory. Cells cultured from donors with EBV antibodies displayed autonomous growth within 30–60 days and have retained unlimited growth potential for >3 years. Although most of these cell lines contain between 0.1–3.0% of cells with detectable EBV antigens, 2 lines have remained "virus free." In contrast to this, none of the cells from 18 EBV seronegative donors or from 15 cord-blood samples could be established in long-term culture, though some survived for up to 120 days.

Direct evidence for a lymphoproliferative effect of EBV was obtained by Pope et al. (*8*) and Gerber et al. (*9*) by demonstrating lymphoblastoid transformation of leukocytes from EBV-seronegative donors after being exposed to cell-free preparations of EBV. This effect could be abolished by addition of antiviral antibody. The virus-transformed cells had an unlimited growth potential in culture, whereas the untreated control cells lost viability after 3–5 weeks. Recently we reported that infection of leukocytes with EBV markedly stimulated cellular DNA synthesis (*10*). As shown in text-figure 1, virus-induced DNA synthesis increased stepwise during 12 days, whereas untreated leukocytes exhibited little or no uptake of ^{3}H-thymidine. Stimulation of DNA synthesis could be inhibited by inactivation of virus infectivity. Autoradiographs demonstrated that up to 20% of EBV-stimulated cells had incorporated ^{3}H-thymidine. These cells increased markedly in number after 20–35 days and attained autonomous growth. By contrast, untreated control cells failed to multiply and gradually degenerated. These results indicate that induction of DNA synthesis in leukocytes from EBV-seronegative donors was due to some biologic activity of EBV rather than a response to antigenic stimulation.

Is the EBV-induced transformation of lymphocytes analogous to malignant transformation observed with oncogenic DNA viruses? Since transplantation to syngeneic hosts was not feasible, we used immunosuppressed, newborn mice to study this problem (*11*). Newborn BALB/c mice were treated within 8 hours after birth with antimouse lymphocyte serum. They were inoculated on the same day intraperitoneally with 10^5–10^8 cells per mouse. The source of the cell inocula is listed in table 2. Cell lines AV_1 and JQ are EBV-transformed lymphoid cells derived from 2 EBV-seronegative donors A and Q, respectively. The EB_3 cell line originated from a Burkitt tumor, and NC_{37} is a spontaneously transformed cell line of nonmalignant origin. The number of virus-containing cells in these lines ranged from <0.01 to 50%. All animals were examined daily for appearance of palpable tumors. They were considered positive if they were moribund or died with grossly identifiable tumors after the first 5 days of transplantation. The results of heterotransplantation experiments in 250 mice are summarized in table 3. No tumors were detected in 40 mice inoculated with buffy-coat cells of donors A and Q. On the other hand, mice inoculated with lymphoid cells died between 14 and 27 days, with a median survival time of 19 days. The 50% lethal doses ranged from 1 to 10×10^6 cells depending on the particular cell line. Autopsy of these animals revealed a grayish, slightly hemorrhagic, gelatinous tumor mass involving most of the intra-abdominal or-



TEXT-FIGURE 1.—Incorporation of ^{3}H-thymidine into leukocytes from an EBV-seronegative donor. Uninoculated control, △– – –△. Cells infected with EBV, ●—●. *Note* absence of stimulation with inactivated EBV, ▲—— ——▲.

gans (fig. 1). Microscopically, these tumors consisted of markedly anaplastic, pleomorphic, and undifferentiated cells (fig. 2). They metastasized to all organs except the heart, invaded blood vessels and lymphatics, and penetrated through the intestinal mucosa. There were perivascular and peribronchial pulmonary invasion (fig. 3) and infiltration of the bone marrow. A few animals showed involvement of the meninges (fig. 4) and brain.

TABLE 2.—*Heterotransplantation of human lymphoid and buffy-coat cells*

Cells	Source	Percent EBV-positive cells
AV$_1$	EBV-transformed lymphoid cells of donor A	30–50
JQ	EBV-transformed lymphoid cells of donor Q	<0.5
A	Buffy-coat cells of donor A	0.0
Q	Buffy-coat cells of donor Q	0.0
EB$_3$	Burkitt's lymphoma cell line	3–5
NC$_{37}$	Spontaneous, transformed lymphoid cells of normal donor	<0.01

TABLE 3.—*Result of heterotransplantation of human lymphoid and buffy-coat cells*

Cells	Type	Source	50% lethal dose
AV$_1$	Lymphoid	Donor A	1×10^7
JQ	Lymphoid	Donor Q	5×10^6
EB$_3$	Lymphoid	Burkitt	1×10^6
NC$_{37}$	Lymphoid	Normal	1×10^7
A	Buffy-coat	Donor A	—
Q	Buffy-coat	Donor Q	—

The tumors produced by these 4 lymphoid cell lines had essentially similar histopathologic features, and the degree of metastasis and invasiveness was nearly the same. The tumor cells had a human chromosome complement and displayed intracellular and membrane immunofluorescence when tested with EBV antibodies. The in vivo activity of these cell lines was independent of the origin of the culture or the number of cells with detectable EBV. These results suggest that both autonomous growth in culture and invasive growth in a heterologous host are related to the presence of the EBV genome.

The presence of the EBV genome in "virus-free" lymphoid cell lines was demonstrated by several investigators by the following experimental approaches:

1) Clones derived from single cells gave rise to virus-positive progeny (*12*).

2) Viral DNA was detected by nucleic acid homology studies (*13*).

3) EBV-associated, soluble complement-fixing antigens were demonstrated (*14–16*).

4) The viral genome could be activated by treatment with 5-bromodeoxyuridine (BUDR) (*17, 18*). An example of BUDR-induced activation of EBV is demonstrated in figures 5 and 6. After treatment with 10–20 μg of BUDR for 6–8 days, up to 10% of the NC$_{37}$ cells synthesized EBV antigens in contrast to the untreated controls.

A summary of the evidence presented here is given below:

Characteristics of EBV

1) Induction of cellular DNA synthesis
2) Morphologic transformation of human lymphocytes
    a) Cell aggregation or colony formation on feeder cells
    b) Unlimited growth in vitro
    c) Invasive tumors in suitable animals
3) Transformed cells containing little or no virus
4) Viral genome in all cells, inducible by BUDR

It is evident that EBV-induced transformation of human lymphocytes is characterized by changes associated with malignant transformation of mammalian cells by oncogenic DNA viruses. EBV activates the capacity of lymphocytes to grow autonomously in culture and to invade, metastasize, and kill a heterologous host on heterotransplantation. Virus-infected cells appear to be released from the normal regulatory control mechanism as indicated by the activation of host DNA synthesis.

Although there is no direct evidence supporting a causal relation of EBV to human neoplasia, the unique biologic properties of EBV suggest a role other than that of a mere passenger.

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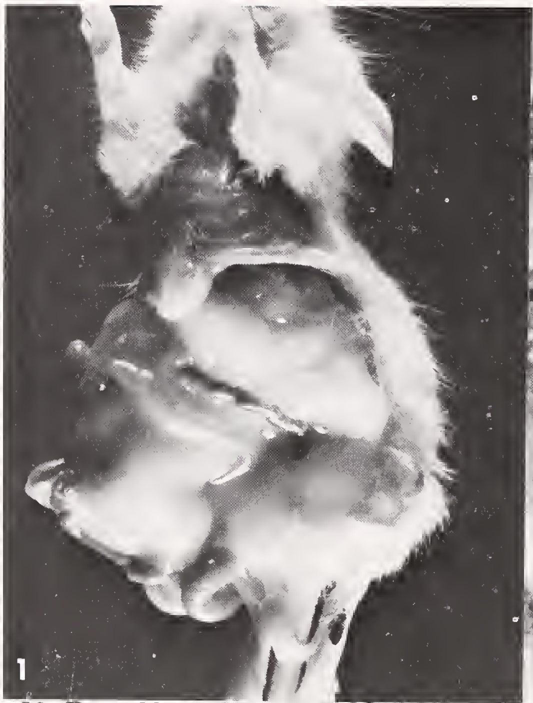
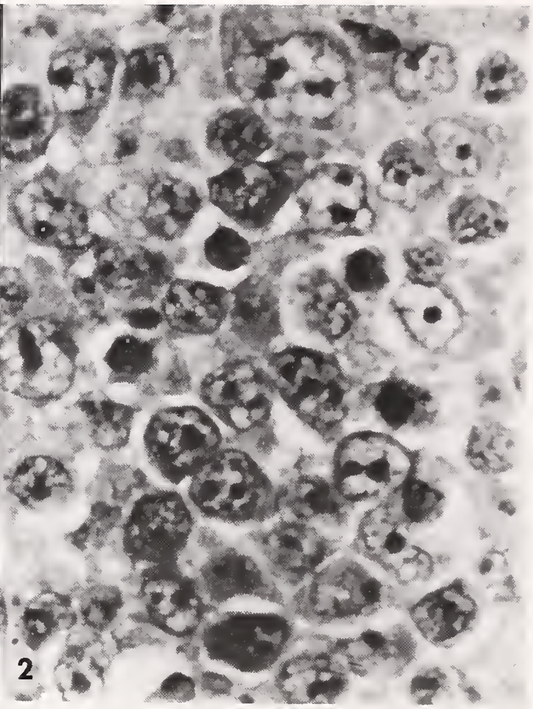
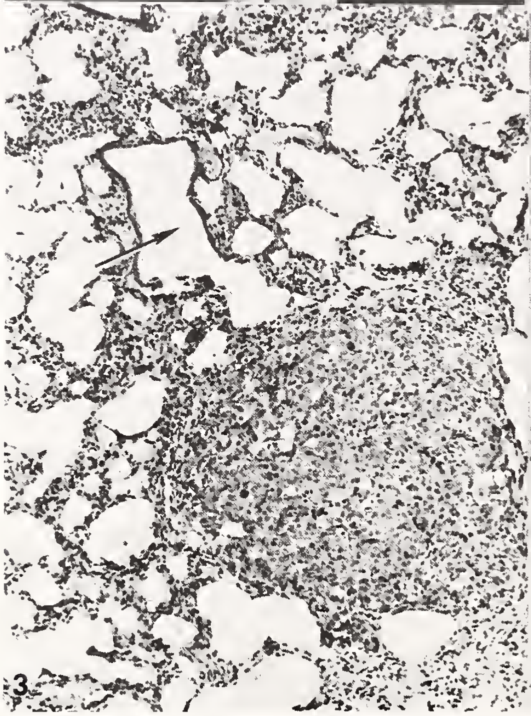






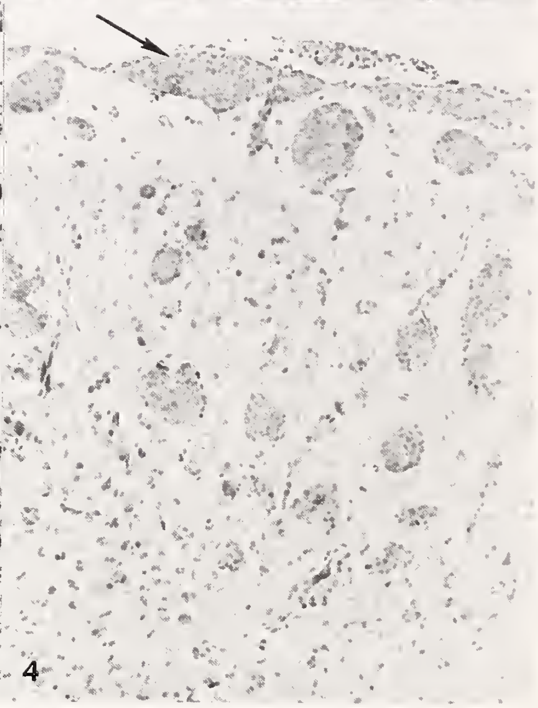
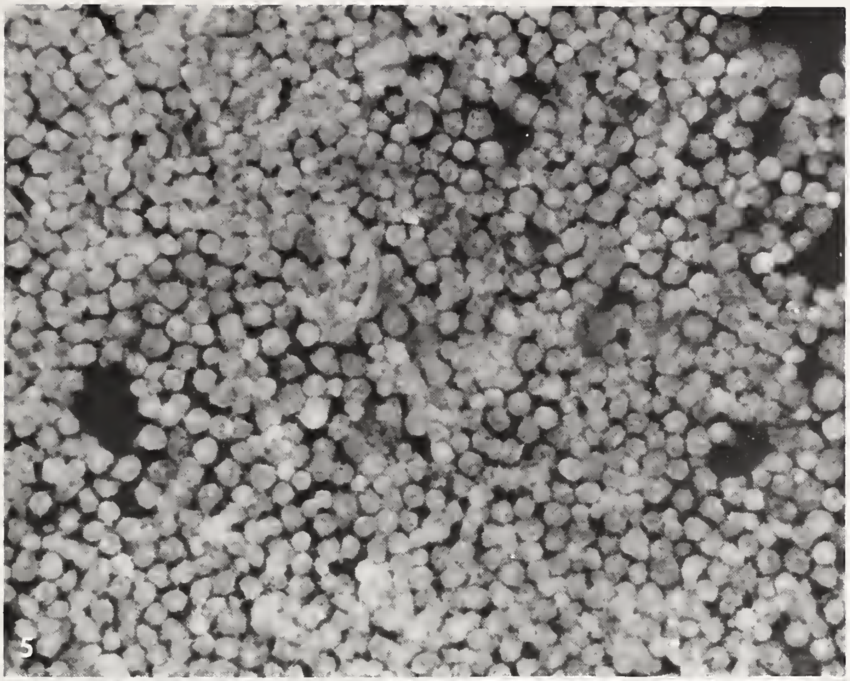


Figure 1.—Gelatinous mesenteric tumor mass enveloping and invading abdominal organs.

Figure 2.—Highly pleomorphic, anaplastic, undifferentiated lymphoid cells from mesenteric tumor in mouse inoculated with AV_1 cells. × 170

Figure 3.—Intrapulmonary nodule of AV_1 cells growing around terminal bronchiole (*arrow*). × 100

Figure 4.—Meningeal (*arrow*) and cortical perivascular invasion by AV_1 cells. × 130



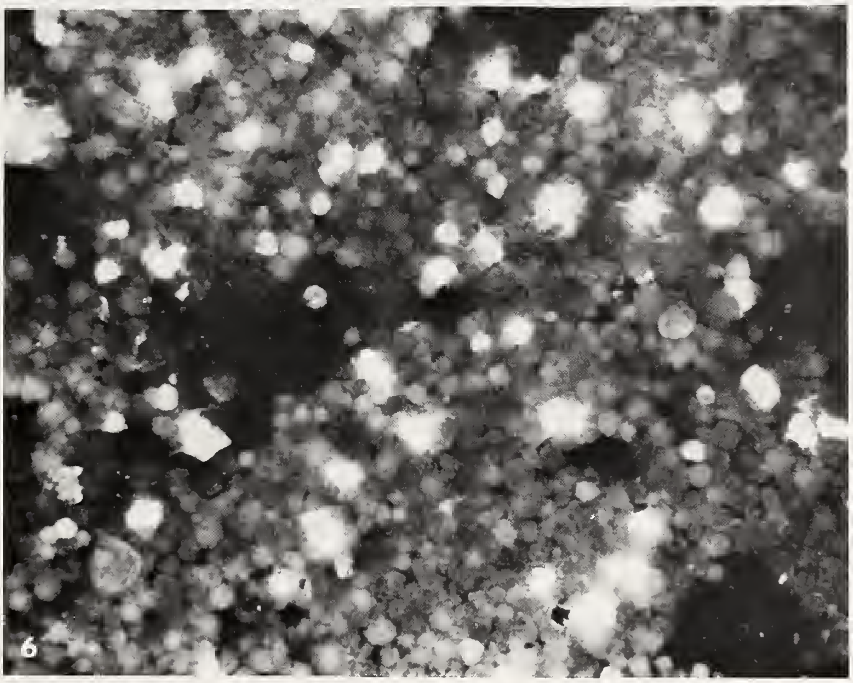


FIGURE 5.—Untreated NC_{37} cells, acetone fixed. *Note* absence of immunofluorescence. × 100

FIGURE 6.—BUDR-treated NC_{37} cells, acetone fixed, showing many immunofluorescent cells. × 100

Virology and Serology—New Approaches to Identification of an Etiologic Agent[1,2]

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SUMMARY—The reasons for suspecting that at least some human tumors have a viral cause are briefly considered, together with some of the difficulties inherent in establishing a causal relationship for any candidate human tumor virus. Experiments are described designed to see whether conventional cultures of normal human embryonic cells could be "transformed" by Epstein-Barr virus as an indication of its possible oncogenicity. Human embryo fibroblasts have been exposed to the virus in suspension and subsequently treated with inactivated Sendai virus in an attempt to assist Epstein-Barr viral penetration by the induction of cell fusion. A cell line has been obtained from human embryo fibroblasts treated in this way which shows many features of "transformation," and the biologic characteristics and significance of these cells are discussed.—Natl Cancer Inst Monogr 36: 73–77, 1973.

AT THE present time we not only accept that a wide variety of animal tumors in many different species are caused by viruses, but also generally agree that at least some human tumors may be caused by viruses. The fact that this International Symposium on Hodgkin's Disease has an entire session devoted to "Virology and Serology" clearly exemplifies this trend in the climate of scientific and medical opinion.

The main advances over the last decade demonstrating a virus causation for an increasing number of animal tumors do not require stressing, and it is well known that there are a number of human malignant tumors currently thought likely to be virus induced. Similarly, 1 or 2 viruses closely associated with human malignant disease are strong candidates as likely human tumor viruses.

All this is most encouraging, but with a suspected human tumor virus we come up against very difficult, special problems. In the animal situation, inoculation and other experiments can be undertaken to prove whether a suspected agent can indeed cause cancer, but with a suspected human tumor virus such work is obviously impossible. Thus, once indirect and circumstantial evidence has built up to suggest that a given virus should be suspected of oncogenicity in man, direct proof of its role in the causation of malignancy is likely to be impossible to obtain by the usual means; there are great difficulties in devising experiments conclusively to establish a carcinogenic role, and this is particularly so where animal hosts are lacking.

It is necessary, therefore, to decide on the value of accumulating more and more evidence for the association of a given agent with a given human malignancy, since however much information of this type is obtained, it cannot give a final definitive answer, and at some stage a new and more dynamic approach must be considered. Elaborate seroepidemiologic surveys have been proposed (*1*), and evidence strongly suggesting a

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] This investigation was aided by the Cancer Research Campaign. One of us (M.P.) is in receipt of a scholarship for training in research methods from the Medical Research Council, London.

viral cause might be obtained if development of a particular type of tumor were always to be accompanied by seroconversion in relation to the same virus. Such a clear-cut association is found with infectious mononucleosis and Epstein-Barr virus (EBV) (*2, 3*); but for oncogenicity it might be necessary for a widely infecting virus to be present for months or years in a specially susceptible individual before a tumor is initiated. In such a case seroepidemiology, even on the largest scale, will not provide the answer.

It seems that at present the only way to resolve this difficulty is by the long-term development of an experimental vaccine, initiation of a pilot trial vaccination program, and the prospective surveillance of the vaccinated population to determine any consequential decrease in the expected number of tumors. With such a procedure, it should be possible to explore directly the suspected connection between the vaccine agent and tumor incidence; in the case of EBV, now widely suspected as a human tumor virus perhaps causing Burkitt's lymphoma, the special features both of the virus and this tumor which favor such an investigation have recently been pointed out (*4*).

From contributions to the present Symposium, it seems possible that Hodgkin's disease may well prove to be an infectious condition (*5*), and there is tenuous evidence that EBV is at least associated with some cases of the disease (*6*). If EBV is seriously to be considered as a possible human tumor virus, we are rapidly reaching the stage where new approaches are needed to investigate its oncogenic function.

Although successful vaccination control has already been achieved with a herpesvirus-induced tumor in animals (*7, 8*), there are formidable problems in the preparation and administration of an EBV vaccine for human use. As an alternative, it was considered that information on the potential carcinogenicity of EBV might be obtained from investigations on the transforming ability of EBV for human fibroblasts in vitro. It has been known for some time that EBV can infect human lymphoid cells (*9, 10*) and induce changes suggestive of malignant transformation (*11, 12*). On the other hand, it has also been known that conventional infection of monolayer cultures with EBV is not possible (*13*); it was thought, however, that some unusual experimental procedure might allow infection to take place and that the infected monolayer cells might provide conventional transformed cultures for the investigation of EBV-induced malignant change by techniques worked out with cells transformed by known DNA animal tumor viruses.

METHODS AND OBSERVATIONS

Human embryo fibroblasts have accordingly been treated with EBV, not as a monolayer but in suspension; thereafter some were also mixed with inactivated Sendai virus to facilitate entry of any adsorbed EBV by the induction of cell fusion. The experimental methods used in this new approach were detailed in (*14*); the present paper will therefore describe only the characteristics and biologic behavior of the unusual human cell line which has been successfully derived from human embryo fibroblasts exposed to EBV in this way.

Exposure of Fibroblasts to EBV

Partially purified preparations of EBV were mixed in suspension with human embryo skin/muscle fibroblasts, and the virus was allowed to adsorb to the cells for 1½ hours at 37° C in a slowly rotating container. Before being seeded in plastic bottles, the cells were exposed for 90 minutes to ultraviolet-light-inactivated Sendai virus or balanced saline solution. In addition, a sample of the fibroblasts unexposed to EBV was also treated with the inactivated Sendai virus to act as a control.

Emergence and Isolation of Transformed Cells

After 7 days' incubation, the fibroblasts in all bottles had formed confluent monolayers. In those cultures where the fibroblasts had been exposed to EBV in suspension at the start of the experiment, groups of 20–30 large polygonal cells were seen lying within the normal cell sheet (*14*). The groups of polygonal cells increased in size to form macroscopically visible colonies which, by 19 days, were heaped up in the shape of a cone, often with a central crater (*14*). This layered mode of growth was especially well seen in sections cut vertically through the colonies (fig. 1). In the cultures where the fibroblasts had been exposed to Sendai virus in addition to EBV, the number of colonies was about 7 times greater. No polygonal cells were present among the fibroblasts treated only with Sendai virus.

Passage of picked colonies of polygonal cells gave rise to cultures in which such cells outgrew the fibroblastic elements unavoidably transferred with them, so that on further passage a uniform population of polygonal cells was obtained.

Biologic Attributes of Transformed Cells

When viewed alive, the cells were flat, polygonal, and about 40–50μ in diameter. The foregoing features remained evident after the cells were stained. Staining also revealed in most cells rounded or oval nuclei, usually containing 5–8 nucleoli: Multinucleate cells and frequent tripolar and tetrapolar mitoses were seen.

Growth curves were constructed after 9, 14, 20, and 38 passages, and the doubling time of the cells remained remarkably constant throughout, at 27–28 hours.

In addition, cloning efficiencies were determined in both fluid and semisolid media after 5, 14, 16, and 41 passages, and the cells had a relatively high cloning efficiency in soft agar, similar to that in fluid media. Clonally derived sublines had cloning efficiencies both higher and lower than those of the parent culture.

Tests for growth at various concentrations of serum showed that the transformed cells had a greatly reduced serum requirement when compared to that of normal human-embryo fibroblasts. With 1% serum, the fibroblasts hardly grew, but the transformed polygonal cells grew substantially and even grew appreciably with 0.2% serum.

Cytogenetic studies, both on the fibroblasts used in the experiments and on the transformed cells after 5, 12, and 18 passages, indicated that the former had a normal human male karyotype whereas the latter at each passage examined showed marked polyploidy. The chromosome complement of the transformed cells approached the hexaploid mode and suggested that these cells were derived from the fusion of 3 human diploid cells. Karyotyping of 5 cells from each passage confirmed that the transformed cells were a near-hexaploid cell line.

Investigation of DNA synthesis in confluent monolayers by labeling with tritiated thymidine demonstrated that, in contrast to the behavior of human embryo fibroblasts, the transformed cells showed no inhibition of DNA synthesis when cultures reached confluence. Normal fibroblasts showed some 25% of cells in DNA synthesis before attaining confluence and a drop to 6% on attaining confluence, whereas about 45% of the transformed cells were in DNA synthesis both before and after attaining confluence.

Tests for EBV antigens in the transformed cells were carried out with specific antiserum to EBV (*15*); cells were examined from the original colonies and after 17 and 19 passages; but results were negative.

In addition, the fine structural organization of the transformed cells was investigated. Although the cells did not show features characteristic of fibroblasts, in the early passages profuse fiber formation was present at free surfaces (fig. 2). This feature was not present when the cells were sampled later. Similarly, early passages showed unusual aggregations of chromatin and electron-opaque granules in the nucleoplasm which were not present after repeated subculture. All samples examined from both early and late passages showed characteristic aggregations of monoparticulate glycogen in the cytoplasm of many cells. Apart from these special features, the cells were large and undifferentiated and had no other unusual characteristics. Microvilli were abundant on the cell surfaces and showed interdigitation with neighboring cells where plasmalemmae were in contact: In such situations poorly developed junction zones were sometimes seen.

DISCUSSION

The possibility always remains that the large polygonal cells resulted from inadvertent contamination of the original fibroblasts by cells from established continuous human lines. Apart from lymphoid cells, only 2 such lines have been grown in our Department of Pathology (HeLa and HEp-2), and both of these lines and the transformed cells were typed for HLA antigens; the standard tests (through the kindness of Dr. S. D. Nelson) showed no common antigenic reactivity.

It seems that the polygonal cells apparently arose by transformation of the embryo fibroblasts, and this is supported to some extent by the karyotyping. In addition, production of extracellular fibers in early passages (fig. 2) might also indicate a fibroblastic origin.

When first reported, the cells were described as being "morphologically transformed" (*14*). Undoubtedly cells of unusual morphology were seen among the normal human-embryo fibroblasts after exposure to EBV. These cells continued in their original form for 12 months, and, in addition to being morphologically different, have now been shown to possess many features that follow malignant transformation: They have maintained a rapid and steady doubling time throughout their period of culture, form heaped-up, layered colonies, continue to grow vigorously after 50 passages, have a low serum requirement, show high cloning efficiency in semisolid medium, and continue DNA synthesis after achieving confluence. It would seem, therefore, that exposure to EBV has not only changed the morphology of human embryo fibroblasts but also brought about what appears to be malignant transformation.

The question thus arises of the role of EBV in this process. No evidence of viral antigens has so far been found in the transformed cells, but, by analogy with certain cells transformed by DNA animal tumor viruses, this may be difficult to obtain.

The results so far call for further pressing investigations in an effort to parallel classic malignant transformation of animal cells by known oncogenic DNA viruses; if this parallel can be completed with EBV and human cells, it is thought that important indications of an oncogenic potential for this virus in man may be achieved.

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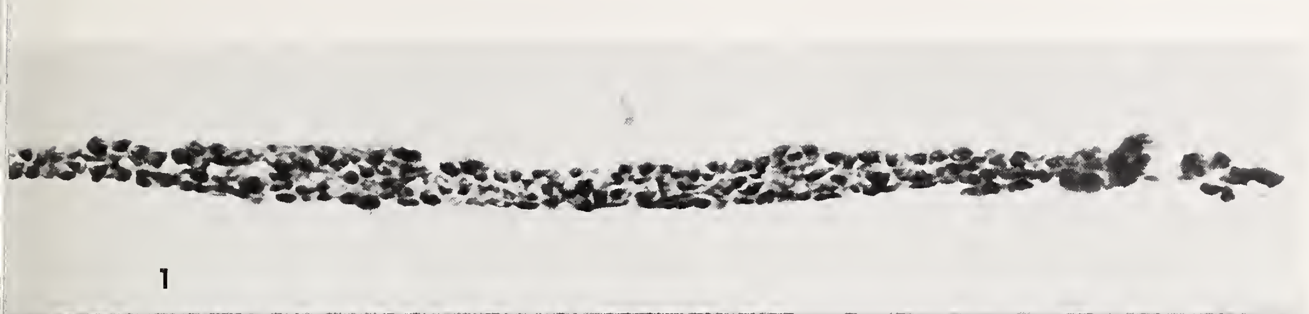

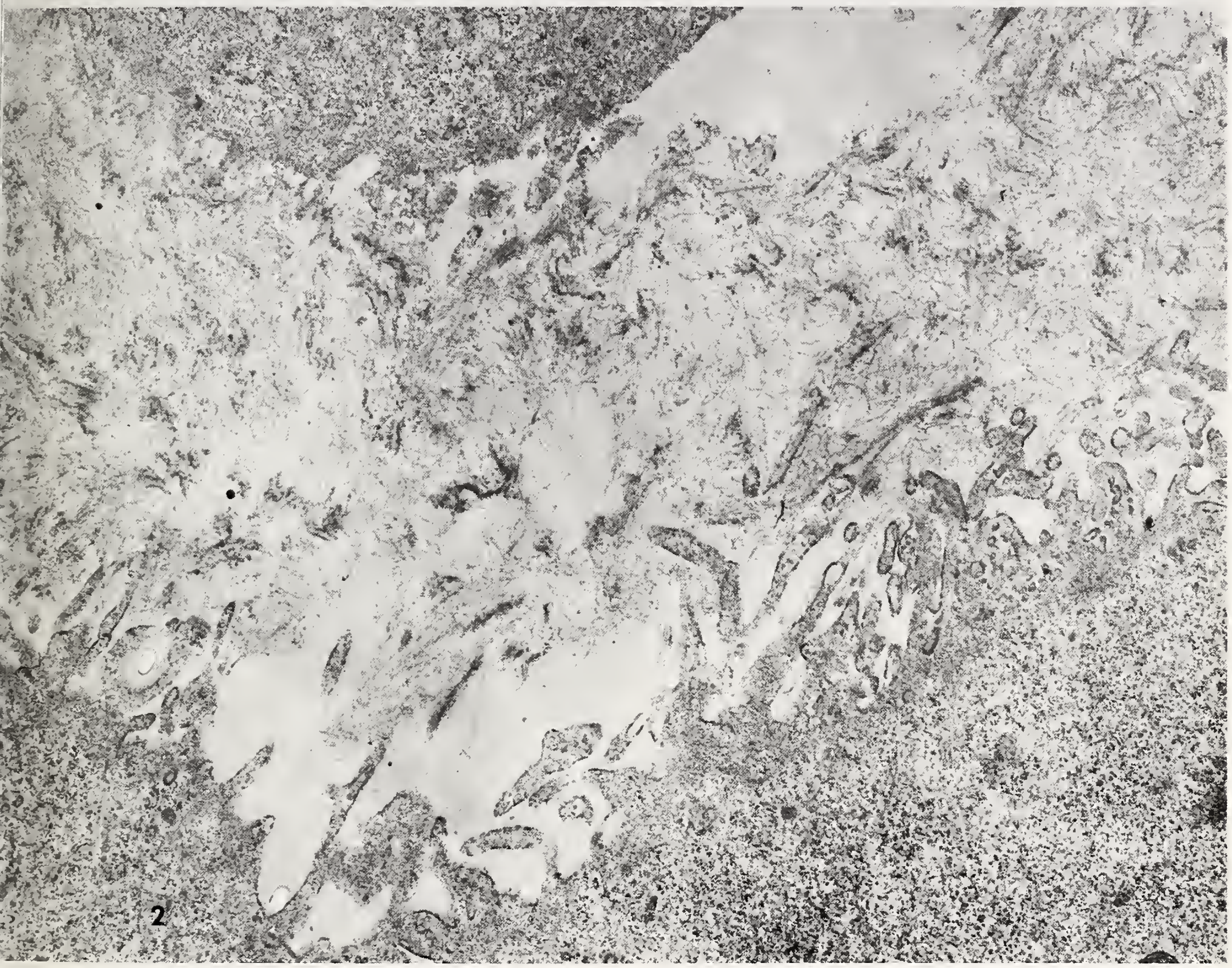

Figure 1.—Section cut vertically through small colony of transformed cells. Heaped-up, layered mode of growth is well seen. Hematoxylin and eosin. × 240

Figure 2.—Election micrograph of thin section cut through an early colony of transformed cells showing small area of 2 adjacent cell surfaces. Intercellular space runs across fiield and is filled with a dense feltwork of fibers. × 20,400

Epstein-Barr Virus-Related Serology in Hodgkin's Disease [1,2]

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SUMMARY—Sera from 489 patients with Hodgkin's disease (HD) and from appropriate controls were examined for antibodies to Epstein-Barr virus (EBV), viral capsid antigens (VCA), and to the D and R components of the EBV-induced early antigen (EA) complex. The results were compared with those obtained in infectious mononucleosis (IM), Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), chronic lymphocytic leukemia (CLL), and corresponding controls. In HD and CLL *not every* patient had antibodies to VCA, but among those who had, there was an overrepresentation of high titers as compared to controls. In IM, BL, and NPC, *all* patients had anti-VCA, most at high titers. While sera with high anti-VCA titers tended to have also anti-EA, the incidences and titers of anti-EA were generally lower in HD and CLL than in IM, BL, and NPC. Anti-EA was directed solely against the D component in IM; with few exceptions solely or dominantly against R in BL; dominantly against D in NPC and CLL; and against R in most, and against D in a few HD patients. The differences in anti-EA responses are unexplained. Based on serologic and other evidence, an etiologic role of EBV seems proved in IM, probable in BL, somewhat less probable in NPC, and unlikely in HD and CLL. It is suggested that immunosuppressive effects of HD and CLL activate latent, persistent EBV infections which boost antibody titers. This does not preclude that primary EBV infections may trigger HD, a possibility deserving statistical evaluation.—Natl Cancer Inst Monogr 36: 79–84, 1973.

THE EPSTEIN-BARR virus (EBV), a member of the herpes group of viruses, was originally discovered in cultured lymphoblasts derived from Burkitt's lymphomas of African patients (*1*). Since then, it has been suspected of being involved in the etiology of this childhood malignancy and, indeed, the evidence supporting this suspicion has been increasing steadily (*2*).

EBV was subsequently detected in lymphoblast cultures derived from peripheral leukocytes of non-African healthy donors and patients with various diseases, and antibodies to EBV were found with generally high frequency in sera of children and adults from all parts of the world. A search for diseases caused by this ubiquitous virus culminated in the discovery that EBV is the etiologic agent of infectious mononucleosis (IM) (*3, 4*). The evidence for this conclusion has been reviewed recently (*5*).

In addition, EBV has been found to be closely associated with nasopharyngeal carcinoma (NPC) (*6, 7*) and, less intimately, with a number of other malignant and nonmalignant diseases on the basis of a greater frequency of high titers of antibodies to EBV-related antigens as

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compared to appropriate controls (*8–13*). Such serologic observations alone do not necessarily imply causal relationships and might find other explanations.

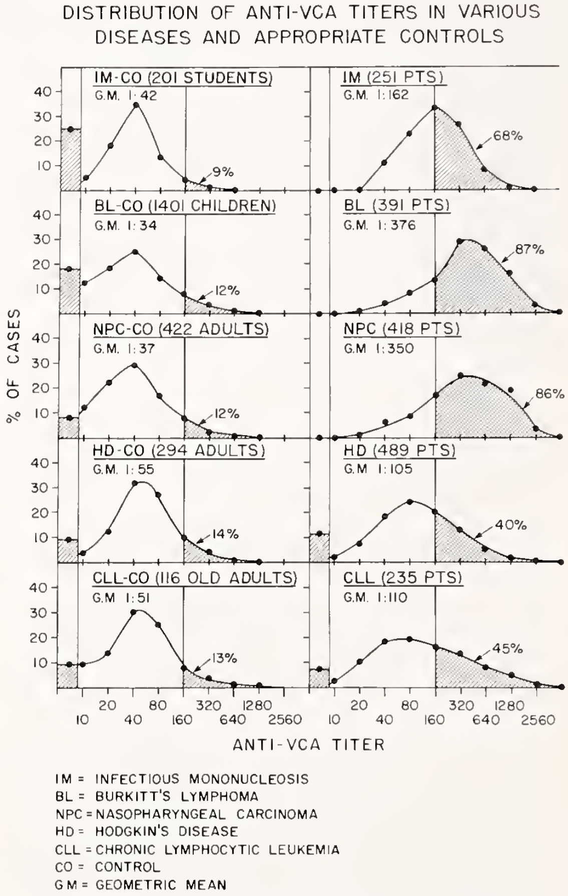
Hodgkin's disease (HD) is among the illnesses often associated with elevated antibody levels to EBV (*8, 9*). Before turning to this disease, we must discuss briefly the antigens involved and the methods used for detection of the corresponding antibodies.

Three major groups of antigens can be differentiated by immunofluorescence: 1) Epstein-Barr viral capsid antigens (VCA) (*14, 15*); 2) EBV-determined cell-membrane antigens (MA) (*16*); and 3) EBV-induced early antigens (EA) (*15*) which are subdivided into the *D* and *R* components (*17*). In addition a fourth EBV-determined antigen, which is neither VCA, MA, or EA, was detected in continuous lymphoblast cultures by complement fixation (*18–20*), and several precipitating antigens were demonstrated in extracts of viral carrier cultures (*6*) which have not yet been identified and may or may not represent additional antigenic components.

Antibodies to VCA are found in sera from all donors previously infected with EBV. They are titrated in indirect immunofluorescence tests with acetone-fixed smears of cells from virus-producing lymphoblast lines (*21*). Antibodies to MA are present in some, but by no means all, anti-VCA-positive sera. They are detected in indirect immunofluorescence tests with live cells from Burkitt tumor biopsies or cultures or, more dependably now, by the blocking effect of test sera on direct staining by fluorescein-conjugated antibodies to MA from donors without demonstrable isoantibodies (*22*). Antibodies to EA are present in most sera from patients with IM, Burkitt's lymphoma (BL), NPC, and occasionally other malignant or nonmalignant diseases. For their measurement, smears are prepared from lymphoblasts which, after experimental exposure to EBV, sustain abortive infections; i.e., EA, which does not depend on viral DNA replication (*23*), is produced, but VCA and virus particles, which require prior viral DNA replication, are not synthesized.

The EA reaction has 2 patterns of immunofluorescence (*17*): 1) Type *D* is characterized by diffuse staining of the nucleus and cytoplasm of abortively infected cells; and 2) type *R* is characterized by straining restricted to a mass in the cytoplasm. *R,* but not *D,* is denatured by methanol fixation, which differentiates between these 2 components as well as the corresponding antibodies. *R,* in turn, is somewhat more resistant than *D* to digestion by proteolytic enzymes, but the procedure is not sufficiently reproducible to be used routinely. The differentiation between *D* and *R* has revealed remarkable, apparently disease-related, differences in antibody patterns which will be discussed later.

To place the EBV-related serology in HD in proper perspective, it is necessary to compare the results with HD with results obtained with other EBV-associated diseases. Text-figure 1 shows the distribution of anti-VCA titers in IM, BL, NPC,



Text-figure 1.—Distribution of anti-VCA titers in patients with various EBV-associated diseases and appropriate control groups.

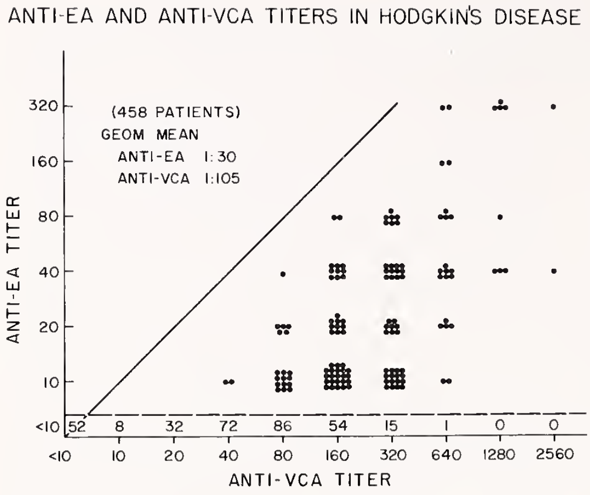
HD, and chronic lymphocytic leukemia (CLL), as well as appropriate control groups. In the first 3 diseases, *all* patients examined showed anti-VCA, most at high titers (≧1:160), and the geometric means ranged from 1:162 in IM to 1:376 in BL. In the corresponding controls, 9–25% of the serum donors, depending on the age range and socioeconomic status, had no antibodies to VCA detectable at serum solution 1:10, or 1:2 in the case of the IM controls, and only 9–14% had high titers. In HD or CLL, *not* every patient showed antibodies to VCA, but there was a definite overrepresentation of high titers as compared to the corresponding controls. The results were remarkably similar, whether the sera were obtained from Swedish, British, or American patients seen by Drs. B. Johansson, G. Hamilton-Fairley, and H. Kaplan, respectively.

The chances of detecting anti-EA improved with an increase in anti-VCA titers (table 1). In IM, about 65% of the patients showed transitory anti-EA responses at titers of ≧1:10. Additional patients had titers of 1:5. In BL and NPC, about 80% revealed anti-EA, depending to some extent on the clinical status and/or stage of the disease. In HD and CLL, 30 and 46% of the patients, respectively, had antibodies to EA. In the control groups, of which only 3 were adequately tested, from 3 to 5% of the serum donors revealed low levels of anti-EA. Aside from the differences in incidence of anti-EA, the geometric mean anti-EA titers of the positive sera varied from >1:100 in BL and NPC, to between 1:30 and 1:46 in IM, HD, and CLL, to ≦1:25 in the controls. Text-figure 2 presents a correlation between anti-VCA and anti-EA in HD, which shows that anti-EA was detectable in few sera with low anti-VCA titers and that the incidence and titers of anti-EA increased somewhat with an increase in the anti-VCA levels.

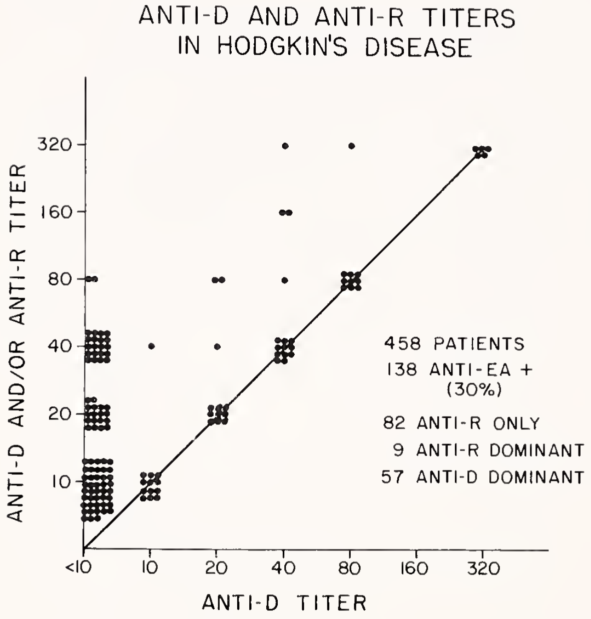
TABLE 1.—*Anti-EA reactions in various EBV-associated diseases and controls (Co)*

Category of donors	Number of patients	Anti-VCA positive Percent	Anti-VCA positive geometric mean	Anti-EA positive Percent	Anti-EA positive geometric mean
IM	203	100	1:162	65	1:33
BL	295	100	1:376	80	1:111
NPC	255	100	1:350	81	1:110
HD	458	89	1:105	30	1:30
CLL	235	93	1:110	46	1:46
IM-Co	201	75	1:42	5	1:17
BL-Co	1401	82	1:34	3	1:16
CLL-Co	116	91	1:51	5	1:25

The differentiation between the *D* and *R* components of the EA complex has led to the detection of remarkable differences in the patterns of the corresponding antibodies in IM, BL, and NPC (*17*). The transitory anti-EA response in IM is almost exclusively of the anti-*D* type. In most BL patients, anti-EA is either solely or dominantly of the anti-*R* type and often detectable for as long as the patients survive. In fact, the anti-*R* reaction appears to be of some prognostic significance in patients who have been brought into remission by chemotherapy. Those who have high anti-*R* titers or are developing these antibodies are prone to have several, ultimately fatal relapses, whereas patients who lack anti-*R*, or who lose these antibodies in time, are likely to become long-term survivors with few, if any, early recurrences (*24*). In NPC patients, anti-*D* is the dominant antibody, although low anti-*R* levels also are usually present. A comparison of the results obtained with NPC patients during active disease, on the one hand, and with long-term survivors on the other, suggests that anti-*D* gradually de-



TEXT-FIGURE 2.—Correlation between anti-VCA and anti-EA titers in HD.



TEXT-FIGURE 3.—Correlation between anti-EA titers determined with methanol-fixed (anti-*D*) and acetone-fixed smears (anti-*D* and/or anti-*R*) of abortively EBV-infected Raji cells in HD.

clines and disappears after successful radiation therapy and thus is useful for monitoring the effectiveness of treatment (unpublished data). In CLL, anti-EA, if found, also is dominantly of the anti-*D* type. In HD (text-fig. 3), two-thirds of the patients with anti-EA have solely or dominantly anti-*R*, but, when the anti-VCA titers are very high, anti-*D* dominates. In control populations, anti-EA is rare and if found, as a rule, it is present at only low titers. If of the anti-*D* type and involving young individuals, it might reflect a recent primary EBV infection, whether or not accompanied by signs of IM. Anti-*R* appears presently to reflect the extent of the latent EBV carrier state which is regularly established in the lymphoreticular system after primary EBV infections.

A review of these and other data clearly shows that, among the so-called EBV-associated diseases, only IM has been firmly established to be etiologically related to EBV (*5*). In BL, and to a lesser extent in NPC, a causative or contributing role of EBV seems likely, even though a passenger role of the virus in BL, and especially in NPC, has not as yet been rigidly excluded. If causally related, undoubtedly other, as yet unknown, factors must operate for tumor induction. In these 2 malignancies, every patient has antibodies to EBV, usually at high titers (*7, 17, 21, 24, 25*), and viral DNA has been detected in every BL and NPC biopsy (*26*). It has not yet been determined, however, whether Epstein-Barr viral DNA in NPC biopsies is restricted to the lymphoid elements of the tumor or present also in the carcinoma cells. Furthermore, EBV was shown to transform lymphoid cells in vitro (*27, 28*), which could account for the development of BL, but attempts to transform other types of cells have failed and thus yielded no support for a causal relationship between EBV and NPC.

The relation of EBV to other malignancies such as HD (*8, 9*) and CLL (*10*) or other nonmalignant diseases, such as sarcoidosis (*11, 12*) and systemic lupus erythematosis (*13*), is likely to be of a different nature. First of all, not all patients with these diseases have antibodies to EBV-related antigens, but among those with anti-VCA, there is a significant over-representation of high titers. If EBV were etiologically involved, it would account for only part of the cases.

Turning to HD, several possible explanations may be entertained. *a*) It has been suggested, but also disputed, that HD is not a single entity and thus EBV could be one of its causes. Indeed, antibody levels to EBV-related antigens are elevated especially in the sarcomatous, lymphocyte-depletion form, less frequently in the granulomatous form, and at the control level in the paragranuloma type of the disease (*8, 9*).

b) Primary EBV infections might precede the development of HD and thus either induce the disease in line with *a*) or trigger it by activation of a latent, as yet unknown, oncogenic virus. Over 50 patients, 3 studied in this laboratory and 48 observed by Dr. Henry Kaplan (personal communication), developed HD within a few years after IM. From 1 patient, sera were tested in the course of his IM, with anti-VCA titers of 1:320 and anti-*D* titers of 1:40, characteristic of this disease. Two years later, after the HD had been diagnosed, the anti-VCA titer was 1:640, the anti-*D* titer 1:20, and the anti-*R* titer 1:320. Unfortunately, it will never be known whether during the 2-year interval the anti-VCA and anti-*D* titers initially declined, as expected in convalescence from IM, or persisted throughout at high levels, or when anti-*R* started to arise. These observations may be anecdotal but indicate the need for a statistically valid follow-up study of IM patients with respect to the incidence of subsequent HD and other lymphoproliferative malignancies.

Finally *c*), the increased antibody levels to EBV-related antigens might be due to the immunosuppressive effects of HD. Since EBV persists after primary infections in the lymphoreticular system, the latent carrier state might be activated by immunosuppressive diseases or therapy, and thus boost antibody levels which would not be affected by immunosuppression. Indeed, this possibility may also explain the elevated antibody levels found in some patients with sarcoidosis and systemic lupus erythematosis. Such effects would obviously be restricted to EBV carriers and, correspondingly, patients who have not been previously infected by EBV could not respond in this fashion. To validate this interpretation it will be essential to correlate the degree of immunosuppression observed in various forms of HD with the EBV-related serology and to determine the titers of antibodies to other viruses known to cause persistent, latent infections: e.g., herpes simplex, cytomegalovirus, varicella, adenovirus, and other viruses. Such efforts are in progress.

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Invited Discussion: Relationship of Titers of Epstein-Barr Virus to Cell-Mediated Immunity in Patients With Hodgkin's Disease [1,2]

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IT HAS now been well documented that antibodies to the Epstein-Barr virus (EBV) are higher in patients with Hodgkin's disease than in normal individuals and patients with other neoplasms (table 1) (*1, 2*). One study (*2*) showed that the high virus antibody level was unique to EBV among the herpesviruses that were tested. When the antibody levels were analyzed according to histologic subtype with the use of the Rye modification of the Lukes-Butler classification (*3*), it was clear that the histology was more important than stage of disease in determining the titer (*2*), with the lymphocyte-depletion and mixed cellularity groups having significantly higher titers than the nodular sclerosis and lymphocyte-predominance groups.

TABLE 1.—*Antibodies to EBV in patients with Hodgkin's disease or other tumors and in normal controls*

Diagnosis		Mean age (yr)	Number tested	Percent patients with VCA titer >1:640	Geometric mean titer
Hodgkin's disease	(1)*	30.4	63	34.9	1:367
	(2)†	24.6	64	53.1	1:564
Other lymphomas	(1)	34.8	42	11.9	1:132
	(2)	16.9	44	15.9	1:85
Nonmalignant pathology	(1)	27.7	172	3.5	1:162
	(2)	11.4	95	18.9	1:62
Nonlymphoid solid tumors	(2)	19.0	65	30.8	1:80
Normal controls	(1)	37.6	85	4.7	1:91
	(2)	24.1	291	21.6	1:170

* (1) represents data accumulated from patients and controls studied between July 1968 and June 1970 (*2*).
† (2) represents data accumulated from a second series of patients and controls studied between July 1970 and December 1971.

Since the EBV titers were highest in the histologic subgroups classically associated with poor cell-mediated immunity, the possibility of a relationship between delayed hypersensitivity and humoral antibody must be considered. Our

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current studies have been directed toward the correlation of EBV antibody levels to cell-mediated immunity in patients with Hodgkin's disease with the use of standard and EBV-related antigens.

Antibodies to the EBV viral capsid antigen (VCA) (*4*) and early antigen(s) (EA) (*5*) were measured by immunofluorescence according to methods previously described with the use of the HR1K clone of P3J (P3HR-1) as the source of VCA antigen and Raji cells infected with P3HR-1 virus as the source of EA antigen. In each study, the sera from the Hodgkin's disease patients and controls were titered simultaneously without knowledge of diagnosis of the patient or clinical information.

The first series of patients, studied in collaboration with Dr. Hesse and her coworkers in Copenhagen (*6*), included 15 patients with Hodgkin's disease, 14 patients with chronic lymphocytic leukemia (CLL), and 13 controls with cardiovascular disease. Untreated patients and controls were sensitized to keyhole limpet hemocyanin and *Brucella abortus*, and measurements of EBV antibody were made before treatment. While the geometric mean titers (GMT) of the Hodgkin's disease patients were higher than those of the CLL patients and controls, the reactivity to the EBV VCA or EA in the anergic group was no higher than in the reactive group (table 2). There was an indication, however, that EBV titers and delayed hypersensitivity were independently related to prognosis in the Hodgkin's disease patients. Older patients had a uniformly rapid progression of disease, but in the 9 patients <50 years old, both short-term survivors had elevated VCA titers, while only 1 of 7 patients who are still alive had elevated VCA titers. EA titers were significantly lower in the 6 surviving young Hodgkin's disease patients (GMT=1:7) than in the young nonsurvivors (GMT=1:640) despite the small numbers ($P<0.05$). The data are consistent with the findings in our previous study (*2*) in which all 7 patients with low VCA titers ($\leq$1:10) were free of disease after 2 years in contrast to the lower survival rate (9/20) in patients with high VCA titers. In this current study, a failure in the sensitization procedure was also associated with disease activity in both Hodgkin's disease and CLL.

In an investigation with Dr. Elliott Perlin at the National Naval Medical Center and Dr. Paul Gerber of the Division of Biologics Standards, Hodgkin's disease patients were studied by skin test, lymphocyte stimulation, and humoral antibody levels. The lymphocytes were stimulated by phytohemagglutinin (PHA), pokeweed mitogen, and by an EBV preparation adapted by Dr. Gerber to measure specific immunity to this virus (*7*). Of 10 patients, 3 were anergic to a battery of standard skin test antigens (mumps, Monilia, purified protein derivative, trichophyton, histoplasmin, and Varidase, 1:500 and 1:50), but there was no obvious difference in EBV titers between the reactive (2 of 6$\geq$1:640) and nonreactive (2 of 4$\geq$1:640) groups. Lymphocyte-stimulation tests also did not correlate with skin reactivity. Of particular interest was the fact that lymphocytes from 12 of 14 Hodgkin's dis-

TABLE 2.—*Summary of EBV antibody titers in relationship to skin reactivity in Hodgkin's disease patients and controls*

	Reactive			Anergic		
	Number tested	GMT		Number tested	GMT	
		VCA	EA		VCA	EA
Hesse et al. (*6*)	34	1:133	1:8	8	1:147	1:12
Perlin et al.	6	1:77	1:4	4	1:269	1:4
Levine et al.	8	1:71	1:2	0		
Total	48	1:112	1:6	12	1:180	1:8

ease patients failed to react with EBV, although only 5 were unreactive to pokeweed and PHA. The lack of correlation between the tests of cell-mediated immunity is not surprising in view of similar results in acute leukemia (*8*), but it will be most important to follow patients through the course of disease before any definite conclusions can be reached.

A study has been initiated recently in collaboration with Dr. Ronald Herberman of the National Cancer Institute in which skin tests are being used to relate delayed hypersensitivity to antigens associated with EBV and Hodgkin's disease with EBV titers. The skin test antigens associated with Hodgkin's disease are derived from the patient's own tissues (*9*), while the EBV-associated antigens are prepared by harvesting the membrane fractions from cell lines that are monitored for EBV-associated membrane antigens by immunofluorescence (*10*). With the 8 lymphoma patients tested thus far, 6 with Hodgkin's disease and 2 with American Burkitt's lymphoma, advanced disease was associated with lack of reactivity to EBV-associated antigens in spite of reactivity to standard antigens, but it is still too soon to be certain of the specificity of the skin test results or to correlate skin tests with EBV titers or survival.

In an earlier study of patients with CLL (*11*), we found significantly elevated EBV titers in patients with minimal disease and suggested that the titers reflected a predisease event rather than immunodepression. Similarly, the evidence to date in 32 Hodgkin's disease patients and 17 patients with other malignant lymphoproliferative diseases indicates that high EBV titers do not reflect general immunologic depression, though delayed hypersensitivity and EBV antibody titers independently seem to be of prognostic importance. Whether there is a relationship between specific EBV humoral and cell-mediated immunity will require further studies with the use of a variety of in vitro tests and skin tests.

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SUMMARY OF INFORMAL DISCUSSION ON VIROLOGY AND SEROLOGY

P. H. Levine commented that in a recent study at the National Cancer Institute Doctors O'Conor and Berard independently reviewed the slides from a series of American Burkitt's lymphomas. It was of interest that all patients with typical American Burkitt's lymphoma were Epstein-Barr virus (EBV) positive. The age relationship was also important in that the young American patients with Burkitt's lymphoma had a geometric mean titer of 1:425 whereas those of the young controls (the normals) and those with acute leukemia were 1:7 and 1:11. Thus, in all these diseases it is very important to break down all parameters, e.g., the histology, the age of onset, etc., so that one can clearly distinguish between the clinical and the laboratory data.

R. A. Good asked Doctor Levine whether the lymphocyte-stimulation studies had been performed on isolated lymphocyte populations or on direct cultures. He considered this extremely important to the interpretation of the data. **Levine** replied that the lymphocyte-stimulation work, performed by Doctors Pearl and Green at the National Naval Hospital, involved the use of isolated lymphocyte populations.

H. S. Kaplan referred to Doctor Stevens' statement that there was evidence in areas in which insect abatement had been utilized that the incidence of Burkitt's lymphoma goes down, and asked him to comment further on the nature of that evidence and just how convincing it is. He noted that he had been interested for some years in the possibility that an additional approach to the demonstration of an etiologic role for EBV would be the use of randomized patterns of insect abatement in the high endemic areas. **D. A. Stevens** stated that Doctors Wright, O'Conor, and Davies had done such epidemiologic studies in the field and were better qualified to answer the question. **D. Wright** said the evidence relates to the area near Kinshasa and the islands of Zanzibar and Medang off the north coast of New Guinea, in which malaria control has been introduced and which now have less Burkitt's lymphoma than would be expected.

G. T. O'Conor stated he did not doubt the relationship of EBV to infectious mononucleosis (IM), but that Doctor Henle had said that the evidence for the etiologic role is unequivocal. The serologic evidence is certainly very strong and a couple of years ago there were reports that the agent had actually been given to some patients with resultant disease. He asked Dr. Henle to comment on evidence relating to human inoculation. **W. Henle** replied that leukemia patients had been given EBV by Doctor Grace. This work was reported at Atlantic City, New Jersey, but the data have never been published and unfortunately Dr. Grace's death interrupted this work. The question has been whether the antibody was really specific, since no absorption had been done. The only convincing evidence comes from the 3 open-heart surgery cases in which the post-transfusion syndrome was definitely due to EBV. Quite recently they have been able to use gibbons without antibodies to EBV. First they produced lymphoblast lines by cocultivation with Burkitt's tumor cells, and regularly could obtain gibbon lymphoblast lines. These lines were given to the EBV-negative animals in the hope that tumors might develop but, unfortunately, the cells were destroyed even though some attempts were made at immunosuppression. However, the animals were converted from negative to positive within 7 or 10 days. This suggested that, if we give too much virus, we get antibodies too rapidly and, therefore, any disease which might be developing would be interrupted. In subsequent experiments, virus was given by intranasal spray but did not yield any infection. In the next 3 experiments, animals were bled the day before, their lymphocytes were exposed to EBV in vitro, and then the cells were given to 3 animals intravenously. All 3 animals were converted after an incubation period of >4 weeks. Antibodies appeared in the 5th week. One animal showed no clinical response, but 2 of the animals showed lesions in the tonsils from the 5th to the 7th week which were minor but, nevertheless, gave some indication that the gibbon might be a good animal for further trials. Everything we now know about the relationship of EBV to IM fits the picture that this is the cause of IM. The burden of proof is now with the doubters.

Part 3. Immunology

Chairman: Robert A. Good

INTRODUCTION

In my opening remarks, I want to review some of the terminology and concepts of modern immunobiology. We are still in a state of great flux and rapid development in this field, but we have made much progress in the last 10 or 12 years. Really the beginning of the 2-component concept of the immunologic apparatus goes back to the mid-1930's when it became obvious that patients with Hodgkin's disease are susceptible to a certain set of infections. The organisms particularly troublesome to these patients were the tubercle bacillus, a variety of fungi, and, in the late stages of the disease, certain virus infections. At about the same time, patients with myeloma were observed to be very susceptible to infection but to have very different problems with infections. They developed recurrent pneumonia due to the pneumococcus, *Hemophilus influenza,* and other encapsulated bacteria such as pseudomonas. Thus, 2 different sets of pathogens were troublesome to these 2 types of patients with lymphoreticular malignancies.

No real explanation for these differences was forthcoming until the allergist, Schier, observed in 1956 that patients with Hodgkin's disease frequently are anergic; i.e., they cannot express cell-mediated immune responses normally to a battery of antigens. I can illustrate this with some old data that we developed at the University of Minnesota in the mid-1950's. We used a battery of skin-test antigens known to elicit a high frequency of responses in immunologically normal persons. We found that more than 50% of patients with Hodgkin's disease whom we considered to be in good condition failed to respond to the entire battery of 6 antigens, each of which produced a high-frequency response in the general population. In this respect, patients with Hodgkin's disease differed from those with other malignant diseases. This was a progressive abnormality and, as the Hodgkin's disease progressed, a very high proportion of the patients became unable to respond to this battery of skin-test antigens.

One could also often transplant skin to patients with Hodgkin's disease. In about a quarter of the instances, an allograft of skin would be accepted exactly like an autograft. One could actually demonstrate a female karyotype of the grafted skin in the form of Barr bodies as late as 28 months after transplant in some of these patients. More often, however, such skin allografts were recognized by the patients as being foreign and rejected by a slow reaction which might take months rather than the several days usually required by the immunologically vigorous individual.

Primary immunization with chemical agents known to elicit delayed-type hypersensitivity, such as dinitrochlorobenzene, again frequently revealed anergy. We also found that patients with Hodgkin's disease could not receive a transfer of delayed hypersensitivity anywhere near as regularly as normal individuals could receive such a transfer with peripheral blood lymphocytes. In this respect, patients with Hodgkin's disease were very different from those with multiple myeloma, who failed to make antibodies well but could reject skin grafts vigorously and could develop very well delayed-type hypersensitivity with primary stimulation. However, patients with multiple myeloma could not form circulating antibodies, whereas those with Hodgkin's disease formed antibodies well to many antigens.

The striking immunologic differences between these 2 diseases were again noted when agammaglobulinemic patients could be studied. Patients with the Bruton type of X-linked infantile agammaglobulinemia cannot form antibodies to any antigen. These patients are markedly deficient in all immunoglobulins; yet they can express very well delayed-type hypersensitivity and allograft rejection reactions. The infections they are susceptible to are like those of the patients with myeloma and unlike those of the patients with Hodgkin's disease.

Laboratory studies of the basis for these different responses became possible when we discovered that the thymus is essential to the development of immunity. An animal that has had its thymus removed in the neonatal period will have a good population of lymphocytes in the peripheral cortex areas, will be able to develop germinal centers well after antigen stimulation, and will have a good population of plasma cells capable of secreting immunoglobulins in the medullary cords, but it will be virtually devoid of thymus-dependent cells in the deep cortical region of the lymph node. From such experiments, we now believe that there is not just a single immunity system but that there are

2 immunity systems: one dependent on the thymus for its differentiation and the other dependent on another differentiation influence. The site of this second differentiation influence is known in birds: the bursa of Fabricius. One can make chickens agmmaglobulinemic by just removing the bursa of Fabricius early enough in development. Such animals will not develop the ability to form plasma cells or to form what we will call the B-lymphocytes that have immunoglobulins at their surface. We can prove the importance of traffic to thymus and bursa by using karyotype markers to trace the movement of these cells and also by using surface isoantigenic markers in mice and chickens. It has been possible to show that yoke sac cells and fetal liver cells can act as prethymic cells and can differentiate into a postthymic, immunocompetent population capable of responding to antigens with lymphokine production. Yolk sac and fetal liver cells can also represent a prebursal cell which, coming under the influence of the bursa of Fabricius, will differentiate into B-cells capable of producing immunoglobulins.

Recently, the story has been getting rather complicated because we now know there are several subpopulations of both T-cells and B-cells and that, to achieve full immunologic vigor, T-cells and B-cells must interact to achieve a full immunologic response. We still do not yet know the molecular basis of specificity in the T-cell type of immunity. Although many people like to believe that this is due to an immunoglobulin, direct evidence for this has not been forthcoming. The B-cell immunity system is equally complex in its fully differentiated form. It is very clear, at least in the chicken, that, to get to an IgG-producing set of cells, one has to differentiate through an IgM-producing cell. We do not know whether this is necessary for the A, D, or E immunoglobulin production but it probably is.

Based on these clinical and fundamental investigations in our considerations of Hodgkin's disease, we must think about 2 immunity systems, one based on the synthesis and secretion of immunoglobulins and the other involved in cell-mediated immune responses of the delayed-hypersensitivity type. The former system involves the B-lymphocytes, which develop along a separate line of differentiation from the T-cell thymus-dependent population. We now have means of quantifying both the numbers of T-cells and the numbers of B-cells in the peripheral blood and lymphoid system. In addition, we can quantify very precisely the synthesis of the product of the separate subpopulations of B-cells. We can identify the cells of each population by studying certain surface isoantigenic markers in experimental animals, e.g., TL, for different stages of thymus-dependent populations in mice. We are learning to use such isoantigenic markers to identify the populations and subpopulatons of cells in man. These studies have made it possible to define many cases of chronic lymphatic leukemia as monoclonal malignant expansions of a "B"-cell deviation, just as myeloma represents a monoclonal expansion of plasma cells and macroglobulinemia of Waldenström is a monoclonal expansion of one line of IgM-secreting B-lymphocytes. A central question to be addressed by our improving capacity to look at these questions is: What cell is monoclonally deviated and expanded in Hodgkin's disease? And what is the actual basis of the T-cell immunodeficiency in this disorder?

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Circulating Lymphoid Cells in Hodgkin's Disease[1]

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SUMMARY—Many patients with untreated Hodgkin's disease had a circulating lymphoid cell population identical with that found in normal controls under conditions of known antigenic challenge and in patients with autoimmune disease. The change in Hodgkin's disease was related to the activity, but not to the clinical stage, histologic type, or prognosis of the disease. It did not help in predicting splenic involvement, and it was not related to cutaneous delayed hypersensitivity. The significance of this phenomenon has still to be determined.—Natl Cancer Inst Monogr 36: 95–98, 1973.

CELLULAR IMMUNITY is impaired in many patients with Hodgkin's disease, particularly in those with widespread disease, and thus much attention has been focused on the lymphocyte. We have shown that the lymphoid cell population in the peripheral blood in this disease is identical with that found under conditions of known antigenic challenge in man (*1*) and differs from normal blood in 3 ways: 1) The number of large lymphoid cells actively synthesizing DNA increases; 2) the number of medium-sized lymphoid cells with basophilic cytoplasm increases; and 3) the number of plasma cells increases.

We have subsequently attempted to determine the significance of these changes in relation to the activity, the stage, the histology and prognosis of the disease, the involvement of the spleen, the presence of cutaneous delayed-hypersensitivity reactions, and the behavior of the lymphocytes in the mixed lymphocyte reaction. We estimated the percentage of large lymphoid cells in the total blood lymphoid cell population in 3 ways: 1) by differential counting, having stained the cells with Romanowsky's stains; 2) by estimating the number of cells actively synthesizing DNA by autoradiography, and 3) by scintillation counting using tritiated thymidine. Since these 3 methods show complete correlation, throughout this article we shall refer simply to the percentage of large lymphoid cells.

Undoubtedly the presence of large lymphoid cells in the peripheral blood is related to the activity of the disease. Text-figure 1, from the article of Crowther et al. (*1*), shows that, in a group of patients who responded satisfactorily to radiotherapy, the percentage of large lymphoid cells returned to normal; but in 3 patients whose disease continued to advance despite treatment, the percentage remained above normal.

There was, however, no correlation between the stage of the disease at the time of diagnosis and the presence of large lymphoid cells in the

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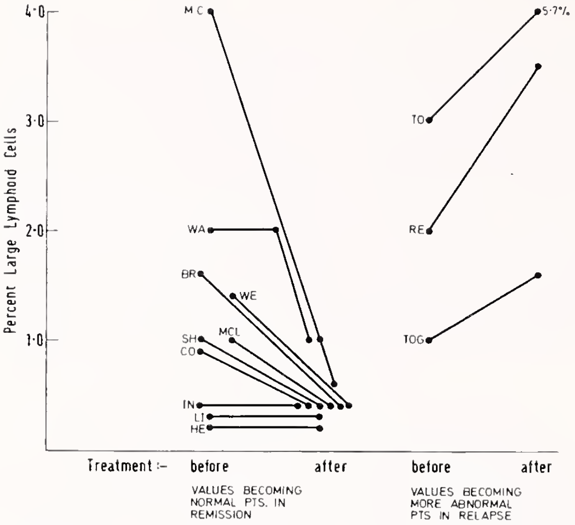
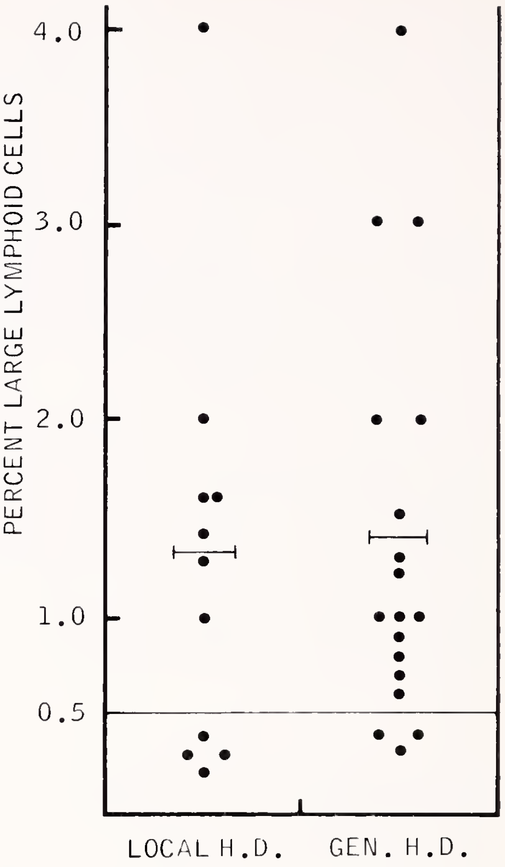


TEXT-FIGURE 1.—Changes in percentage of large lymphoid cells in Hodgkin's disease after treatment with radiotherapy. Blood was taken before and 1 month after treatment. In the first group, the patients all responded satisfactorily to treatment and the lymphoid cells returned to more normal values. In the second group, 3 patients failed to respond and the percentage of lymphoid cells increased [from the article by Crowther et al *(1)*].

blood [text-fig. 2, also from Crowther et al. *(1)*]. The percentage of large lymphoid cells was the same in localized (Stages I and II) as in generalized (Stages II and IV) disease. Similarly, the histologic type did not correlate with the percentage of large lymphoid cells in the peripheral blood.

In a group of 41 patients, followed for 4 years since diagnosis, there was no significant difference between the percentage of large lymphoid cells before treatment and the subsequent behavior of the disease. If one takes 0.5% as the upper limit of normal for the percentage of these cells in the blood lymphocyte population, text-figure 3 shows that: In the group who never had a relapse, 6 of 11 had an increased percentage (55%); in the group who are still alive but have had a relapse, 8 of 11 (73%); and in the group who died, 13 of 19 (58%).

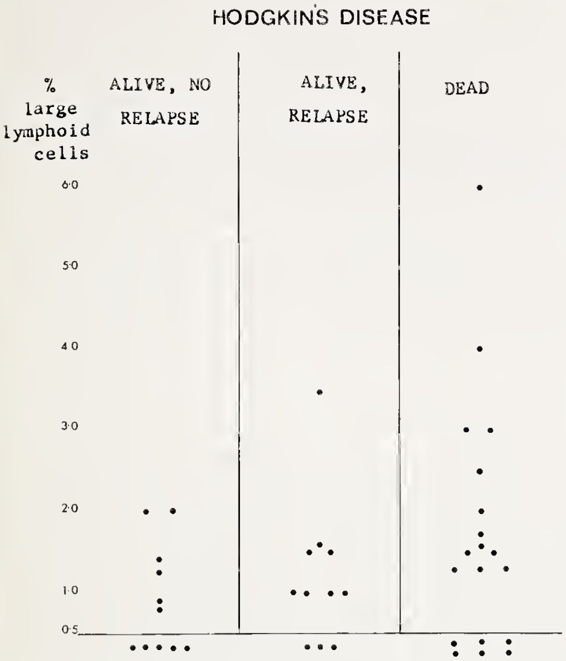
The presence of large lymphoid cells is not related to splenic involvement. In a small series of patients who had a laparotomy and splenectomy, 2 of 7 with splenic invlovement and 2 of 6 without splenic involvement had raised levels of large lymphoid cells (text-fig. 4).



TEXT-FIGURE 2.—Large lymphoid cells in patients with local and generalized untreated Hodgkin's disease, showing no difference between the two groups. The term "local" means Stages I and II, and "general" means Stages III and IV [from the article by Crowther et al *(1)*].

Eighteen patients before treatment were tested with tuberculin and streptokinase/streptodornase for cutaneous delayed hypersensitivity. Dividing the patients into 2 groups, the first being negative to both antigens and the second responding to either one or the other antigen or both, shows that there is no correlation with the percentage of large lymphoid cells (text-fig. 5).

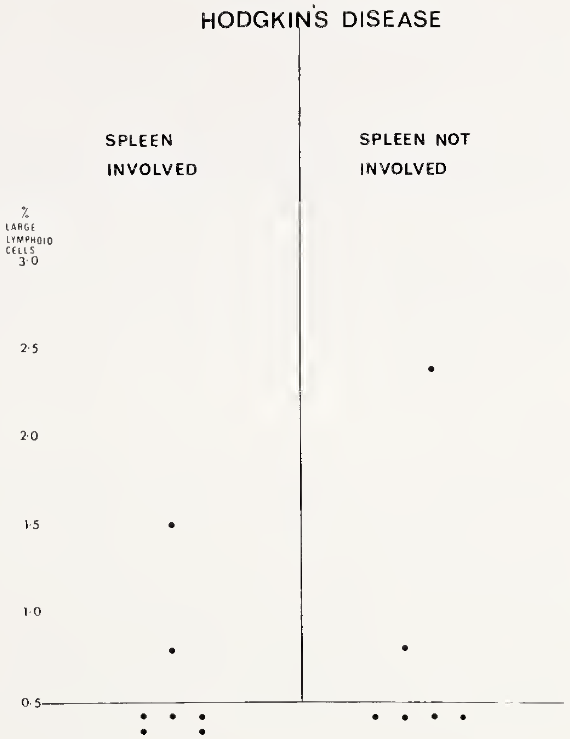
We have recently been interested in the mixed lymphocyte reaction (MLR), and preliminary results have shown that the lymphocytes in 3 of 5 patients studied before treatment reacted normally in the MLR, but 2 failed to do so. Further studies have shown that, during and following radiotherapy, the ability of the patient's lymphocytes to react in the MLR was impaired;



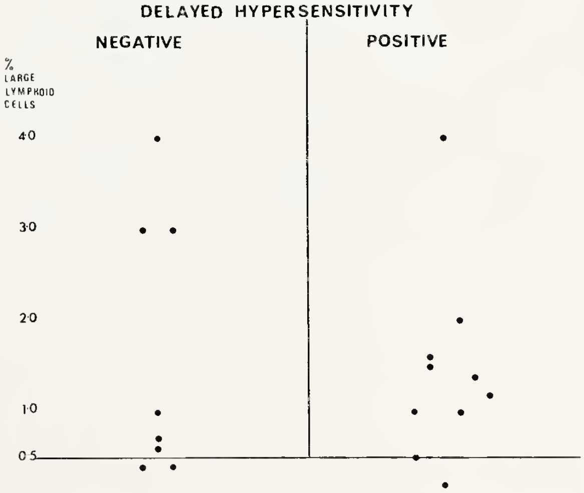
Text-figure 3.—Percentage of large lymphoid cells in the blood lymphocyte population before treatment related to prognosis. There was no difference between the 3 groups.

in addition, in 21 patients receiving quadruple chemotherapy with mustine hydrochloride, vinblastine, procarbazine, and prednisolone (MVPP), the details of which have been recorded by Nicholson et al. (*2*) and in this Symposium by McElwain et al. (*3*), the lymphocyte response was depressed considerably, even after the 4-week period without treatment (table 1). On only 3 occasions did the lymphocytes from the patients respond as well as normal lymphocytes, and 2 of these had received 6 courses of chemotherapy.

Clearly we cannot use this test to obtain useful information to indicate disease activity during treatment. However, it is interesting that in most patients the lymphocytes failed to recover from the effects of chemotherapy, even after 4 weeks without treatment. In acute leukemia, some recovery usually occurs within 2 weeks after intensive chemotherapy (*4*). Failure for this to occur in Hodgkin's disease might indicate that our regimen is excessively "immunosuppressive" or, which is much more likely, that the lymphocytes in Hodgkin's disease are particularly sen-



Text-figure 4.—Percentage of large lymphoid cells in the blood lymphocyte population before treatment related to splenic involvement. There was no difference between the 2 groups.



Text-figure 5.—Percentage of large lymphoid cells in the blood lymphocyte population before treatment related to cutaneous delayed hypersensitivity. Negative group failed to respond to both tuberculin and streptokinase/streptodornase, and positive group responded to one or the other, or both. There was no difference between the 2 groups.

TABLE 1.—*Ability of lymphocytes from patients with Hodgkin's disease receiving MVPP to react in MLR**

Time of testing	Number of patients with: Normal response $>10,000$ cpm	Impaired response 1,000–10,000 cpm	Total inactivity $<1,000$ cpm
4 weeks after 1st course	1	1	3
4 weeks after 2d course		1	1
4 weeks after 3d course			2
4 weeks after 4th course			
4 weeks after 5th course		1	2
4 weeks after 6th course	1	2	2
4 weeks after 6th+ course	1		3
Total No. of patients: 21	3	5	13

* For all cultures, 1 million reacting lymphocytes were suspended in 3 ml of culture medium TC.199 containing 12.5% of serum. A total of 0.5×10^6 stimulating allogeneic lymphocytes treated with 25 μg ml^{-1} of mitomycin C was added. Under these conditions, the stimulating lymphocytes cannot themselves synthesize DNA. These stimulating lymphocytes were obtained from normal donors. These cultures were then maintained in sealed tubes at 37° C for 6 days, after which DNA synthesis in the culture was determined by measurement of the incorporation of radioactive thymidine. A total of 15 μCi of ^{3}H-thymidine (specific activity 15 CimM^{-1}, was added, and after 60 minutes at 37° C the cells were extracted by filtration onto a Millipore filter. Cells on the filter were exposed to 10% ice-cold trichloroacetic acid washed with ethanol, and radioactivity was determined by liquid scintillation counting. Thymidine incorporaton was expressed as cpm. In cultures usng reacting lymphocytes from normal donors, cpm were always $>10,000$. In cultures containing mixtures of autologous cells (normal negative controls), cpm were always $<1,000$.

sitive to treatment. We do not have enough data at present to indicate if this test is useful in determining activity of the disease before treatment but of 5 patients studied it is of interest to note that 3 had normally reacting MLR's. We are now studying this parameter in a much larger series.

From the above data, it is only possible to conclude that the presence of an increased percentage of large lymphoid cells in the peripheral blood is related to the *activity* but not to any other manifestation of the disease. Furthermore, the ability of the lymphocytes to respond in the MLR cannot be used as an index of the activity of disease during treatment.

However, changes in the lymphoid cell population, identical with those known to be present in normal man undergoing antigenic challenge, remain to be explained. There are at least 5 possible explanations:

1) The lymphoid cells are "reacting" against a tumor-associated antigen.
2) They are reacting against some other product of the tumor.
3) They are reacting against a concomitant and as yet unidentified infection.
4) The presence of the cells represents release of cells normally contained within the lymph nodes due to disruption of the lymph nodes, analogous to the leukoerythroblastic anemia associated with bone marrow infiltration.
5) The large lymphoid cells are "malignant," but there is no evidence to support this at the moment.

Whether any of these thories will prove correct remains to be seen.

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In Vitro Evidence of Normal Lymphocyte Function in Some Patients With Hodgkin's Disease and Negative Delayed Cutaneous Hypersensitivity[1]

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SUMMARY—Lymphocytes from patients with Hodgkin's disease were assayed for production of migration-inhibitory factor (MIF) and for thymidine incorporation in response to stimulation by antigen or phytohemagglutinin (PHA). The results were correlated with delayed cutaneous hypersensitivity to the same antigen used in the in vitro studies. From the data obtained, the 26 patients studied could be divided into 2 groups. In group 1 (16 patients), the results of in vitro assays correlated with skin tests. In group 2 (10 patients), in vitro assays were positive though skin test to the same antigen was negative. There were no statistically significant clinical differences between the 2 groups of patients. Lymphocyte response to PHA was normal in the 9 patients of group 2 studied. The finding of a normal proliferative response to PHA and to antigen associated with normal production of MIF suggests that the observed defect in dermal reactivity in group 2 patients may be due either to defective production of other lymphocyte mediators or to a nonlymphocyte host defect. —Natl Cancer Inst Monogr 36: 99–106, 1973.

THE NATURE of the immunologic defect in Hodgkin's disease is not precisely established. The expected incidence of dermal sensitivity to standard skin test antigens is not found in patients with Hodgkin's disease, either at diagnosis or after treatment (*1, 2*). Such patients are unusually susceptible to tuberculosis and mycotic and viral infections and have prolongation of skin graft rejection (*3*). These findings suggest a defect in cellular immunity that could involve lymphocytes, lymphocyte mediators, macrophages, or other as yet unknown host factors. In contrast to the defect in cellular immunity, primary and secondary antibody synthesis is relatively unimpaired in patients with Hodgkin's disease (*4, 5*).

In vitro studies have shown that lymphocytes from some patients with Hodgkin's disease respond abnormally to stimulation by phytohemagglutinin (PHA) and survive less well in culture than normal cells (*6–10*). Aside from study of the proliferative responses to mitogens, specific antigen, and leukocyte antigens, relatively little has been done to evaluate other lymphocyte functions. Holm et al. showed that lymphocytes are deficient in target cell cytotoxicity, and Aisenberg demonstrated that lymphocytes do not function normally in the lymphocyte transfer test (*11, 12*). Except for preliminary observations showing deficiency in interferon and lymphotoxin production by lymphocytes from patients with Hodgkin's disease, nothing is known about mediator production by lymphocytes from patients with this disease (*13, 14*).

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To further clarify the defect in cellular immunity, we studied lymphocytes from 26 patients with Hodgkin's disease and correlated the results of antigen-induced lymphocyte proliferation and production of one of the lymphocyte mediators, migration-inhibitory factor (MIF), with the results of skin tests to the same antigen used to stimulate the lymphocytes in vitro. We found that 10 of the 26 patients studied had normal in vitro lymphocyte function as judged by these 2 assays, but lacked delayed cutaneous hypersensitivity to the same antigen. The dissociation between in vivo skin reactivity and in vitro function has been detected primarily in patients with Hodgkin's disease, rarely in patients with other diseases, and never in normal volunteers.

MATERIALS AND METHODS

Patients.—Twenty-six patients with biopsy-proved Hodgkin's disease were staged according to the Rye classification (*15*). The stage of disease at time of assay represents the most advanced stage demonstrated either by clinical or pathologic criteria before assay. Except for one which could not be obtained, biopsies from all patients were reviewed and classified according to the histologic criteria of Lukes and Butler (*16*). Tables 1 and 2 summarize the clinical information, histology, extent of disease, disease activity, and lymphocyte count at the time of assay.

Skin tests.—Each patients received intradermal injections of first- or intermediate-strength tuberculin purified protein derivative (PPD) and 5 U streptakinase/streptodornase (SKSD). Patients with negative skin tests were retested with second-strength PPD and 50 U SKSD. In addition to these antigens, some normal subjects were tested with Candida, initially at a dilution of 1/100 and, if negative, at a dilution of 1/10. Erythema or induration of >10 mm was considered positive. Negative skin tests in group 2 patients had <5 mm of erythemia and no induration.

In vitro lymphocyte studies.—Lymphocytes were cultured with or without PPD (10 μg/ml) and SKSD (50 U/ml) for the production of MIF and ^{3}H-thymidine (^{3}H-TDR) incorporation. The method for the production of MIF by human lymphocytes was described in (*17*). Supernatants from 3-day antigen-stimulated lymphocyte cultures were concentrated 5 times and assayed for the presence of MIF on normal guinea-pig macrophages in capillary tubes. As previously described, a migration index of $\leq$80% was considered a positive result, indicating MIF production (*17*). Antigen-induced ^{3}H-TDR incorporation was measured in parallel with MIF production. The method used to determine ^{3}H-TDR incorporation was reported (*18*). Six-day cultures were pulsed with ^{3}H-TDR, the DNA-labeled material was obtained, and the counts per minute (cpm) were determined by scintillation counting. The test was considered positive when the incorporation of ^{3}H-TDR in antigen-stimulated cultures was 3 or more times that of unstimulated cultures. Response to PHA was determined by addition of 0.1 ml of PHA-M[5] to a culture containing 1.5x10^6 lymphocytes in medium 199 with 15% autologous serum. When incorporation of ^{3}H-TDR in PHA-stimulated cultures, determined after 72 hours' incubation, was $\geqq$20 times that incorporated in unstimulated cultures, the lymphocyte response to PHA was considered normal.

RESULTS

Both in vitro assays were utilized to test lymphocytes from 21 normal subjects for response to one or more of the following antigens: SKSD, PPD, and Candida. The results of antigen-induced lymphocyte proliferation and production of MIF were correlated with those of skin tests to the same antigens. As text-figure 1 shows, the correlation between in vitro assays and skin test results was excellent. Except for 3 false negative MIF assays and 1 borderline proliferative response, the results of the 2 in

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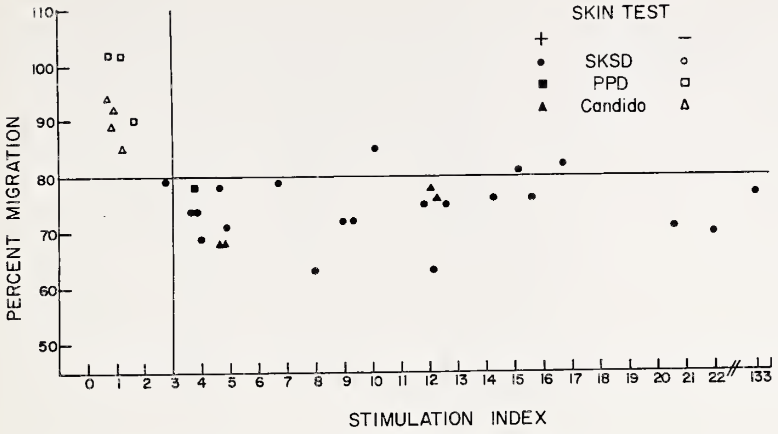
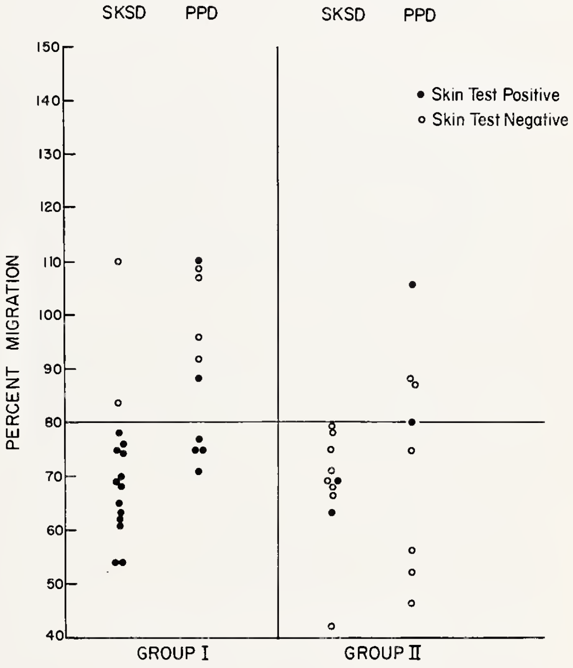
[5] PHA-M was obtained from Difco Laboratories, Detroit, Mich., 48201, and was rehydrated with 5 ml of sterile water before use.



TEXT-FIGURE 1.—Correlation of MIF assay and antigen-induced lymphocyte proliferation with those of skin test to the same antigen in normal subjects. *Solid symbols* indicate positive skin tests. Percent migration of ≤80% and stimulation index of at least 3 constitute positive assays, respectively, for MIF and antigen-induced lymphocyte proliferation.

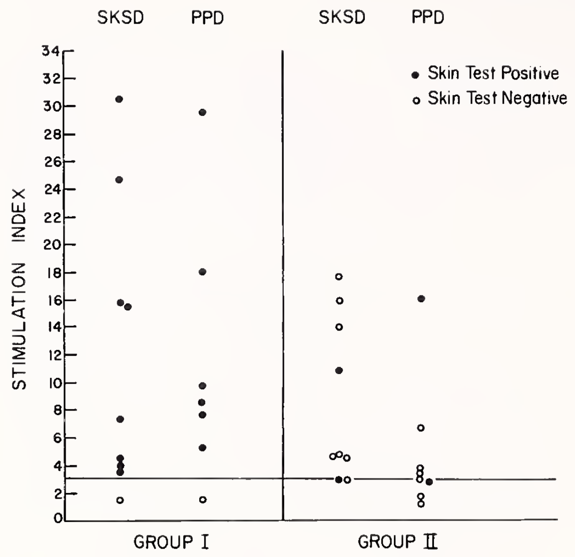


TEXT-FIGURE 2.—Correlation between MIF assay and result of skin test to the same antigen in patients with Hodgkin's disease. Percent migration of ≤80% constitutes a positive MIF assay. In group 2, four patients had a positive skin test to one antigen but a dissociation between the results of the MIF assay and skin test with the other antigen.

vitro assays always agreed with the results of skin tests.

Lymphocytes from all patients were assayed for MIF production in response to stimulation by SKSD or PPD. On the basis of the results, summarized in text-figure 2, these patients could be divided into 2 groups. Group 1 consisted of 16 patients in whom the MIF assay correlated with the delayed cutaneous hypersensitivity reaction to the same antigen, except for 2 instances in which patients with a positive skin test to PPD had a negative MIF assay. In group 2, all 10 patients were skin-test negative to at least one antigen but, in each of these patients, the antigen for which no dermal reactivity was demonstrated stimulated production of MIF. In 4 of the patients of this group, dissociation between a positive MIF assay and a negative skin test was found for 1 antigen. However, in these same patients, dermal reactivity and, in 3 of 4 instances, MIF production, could be demonstrated against the other antigen studied.

The lymphocyte proliferative response to one or both of these antigens were determined in 9 of 13 patients with positive dermal reactivity, in 1 of 3 patients with negative skin reactions in group 1, and in all patients in group 2. The proliferative response of lymphocytes from patients in group 1 always correlated with dermal reactivity to the same antigen. The proliferative response of lymphocytes from patients in group 2 was positive to the same antigen(s) which, though capable of stimulating lymphocytes from group 2 patients to produce MIF, failed to elicit a positive skin reaction (text-fig. 3). Quantitatively the proliferative response to SKSD was the same in both groups. The response to PPD, however, was significantly greater in group 1, with a mean stimulation of 13.3±3.5, than in group 2, with a mean stimulation of 4.2±0.9.

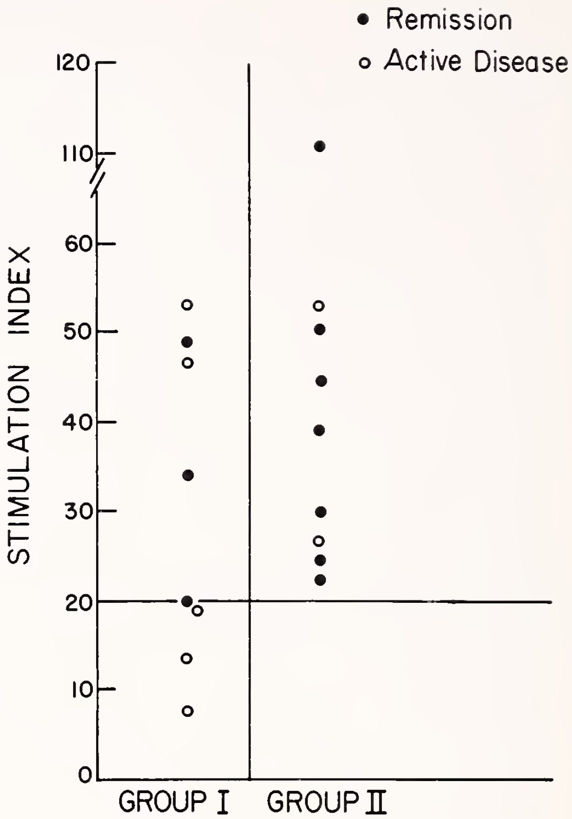


TEXT-FIGURE 3.—Correlation between antigen-induced lymphocyte proliferation and result of skin test to the same antigen in patients with Hodgkin's disease. In group 2, four patients had a positive skin test to one antigen but a dissociation between the results of antigen-induced lymphocyte proliferation and skin test with the other antigen. Stimulation index of ≧3 constitutes a positive assay.

In 3 of the 8 group 1 patients studied, lymphocyte response to stimulation by PHA was abnormal, whereas none of the 9 group 2 patients studied had an abnormal lymphocyte response to PHA (text-fig. 4).

The patients were divided into these 2 groups on the basis of the immunologic findings. However, the clinical features of the 2 groups, summarized in tables 1–3, were very similar. Age of onset, histologic diagnosis, duration of disease, stage of disease, disease activity, and treatment were not significantly different. All patients, except for 4 in group 1, developed Hodgkin's disease during the first major incidence peak in early adulthood. The distribution of absolute lymphocyte counts was similar in both groups, except for a slightly lower mean lymphocyte count in group 2. As reported by others, the average lymphocyte count was decreased with more advanced stages of disease (*1*).

Although the frequency of remission and nodular sclerosis in group 2 did not attain statistical significance, the relatively greater incidence of these 2 parameters might become significant when larger numbers of patients are studied.



TEXT-FIGURE 4.—Results of lymphocyte response to stimulation by PHA in the 2 groups of patients with Hodgkin's disease. A normal response is indicated by a stimulation index of at least 20.

DISCUSSION

Previous experimental and clinical observations support the view that lymphocytes from patients with Hodgkin's disease function abnormally. In vitro studies of lymphocytes from patients with Hodgkin's disease have been primarily concerned with the lymphocyte proliferative response and have shown that, on the average, lymphocytes from patients with Hodgkin's disease have a defective response both to PHA and to specific antigen (*6–10*). Individual patients, however, have lymphocytes that retain a normal proliferative response to PHA independent of the patient's capacity to develop normal skin reactivity. For example, in Aisenberg's study of patients with active Hodgkin's

Table 1.—*Group 1: In vitro tests correlated with results of skin tests*

Patient	Sex	Date of birth	Date of onset	Age (yr)	Histologic diagnosis	Stage at assay	Disease activity at assay*	Lymphocyte count	Treatment
C.M.	M	9/23/41	11–69	29	Mixed cell	I-A	R	965	X-ray
C.R.	F	2/2/41	12–67	26	Mixed cell	III-A	A	388	X-ray
W.J.	M	4/21/96	2–70	74	Mixed cell	III-A	U	650	
J.M.	M	5/4/31	5–70	39	Mixed cell	I-A	U	2480	
J.M.	M	3/28/15	9–70	54	Mixed cell	II-A	U	1120	
R.D.	M	5/4/46	12–69	23	Mixed cell	III-A	U	1087	
M.W.	F	3/3/08	9–65	58	Lymphocyte predominance	I-A	R	1020	X-ray
R.B.	M	1/5/28		27	Lymphocyte predominance	III-B	A	1270	X-ray Chemotherapy
J.M.	M	10/10/40	7–59	18	Paragranuloma	I-A	R	1900	X-ray Chemotherapy
S.A.	M	8/18/45	67	23	Nodular sclerosis	II-A	R	1410	X-ray
L.S.	F	5/6/32	8–65	34	Nodular sclerosis	IV-A	A	516	X-ray Chemotherapy
C.R.	F	1/16/50	6–65	15	Nodular sclerosis	III-A	A	675	X-ray Chemotherapy
D.L.	F	8/6/49	12–69	21	Mixed cell	III-A	U	1740	
F.C.	F	5/18/29	3–60	31	Nodular sclerosis	II-A	R	875	X-ray
M.C.	M	11/19/36	5–69	32	Mixed cell	IV-B	A	1137	Chemotherapy
D.C.	M	11/22/40	1–69	28	Lymphocyte predominance	II-A	R	460	X-ray

* R=remission; U=untreated; and A=active after therapy.

Table 2.—*Group 2: In vitro assays were positive although skin tests to the same antigen(s) were negative*

Patient	Sex	Date of birth	Date of onset	Age (yr)	Histologic diagnosis	Stage at assay	Disease activity at assay*	Lymphocyte count	Treatment
R.H.	M	4/20/33	5–59	26	Mixed cell	II-A	R	1970	X-ray
S.B.	M	1/9/48	4–70	21	Mixed cell	II-A	R	810	X-ray
W.T.	M	10/3/42	1–70	28	Mixed cell	IV-B	R	590	Chemotherapy
E.C.	F	7/30/28	1960	32	Mixed cell	IV-A	A	536	X-ray Chemotherapy
E.S.	M	9/16/45	2–68	22	Mixed cell	III-B	A	430	X-ray
S.G.	M	4/15/01	6–66	64	Nodular sclerosis	III-A	R	625	X-ray
D.W.	M	4/28/26	6–61	25	Nodular sclerosis	II-A	R	524	X-ray Chemotherapy
P.A.	M	2/8/44	8–69	25	Nodular sclerosis	II-A	R	1112	X-ray
W.C.	M	4/1/32	5–65	33	Nodular sclerosis	II-A	R	1200	X-ray Chemotherapy
A.S.	F	6/4/25	4–65	40	Nodular sclerosis	III-A	R	1640	X-ray

* R=remission; and A=active after therapy.

TABLE 3.—*Summary of clinical characteristics of the 2 groups of patients with Hodgkin's disease*

	Group 1	Group 2
Male	10	8
Female	6	2
Average age of onset	33 yr	32 yr
Average duration of disease	3.6 yr	4.7 yr
Average lymphocyte count (patients with positive in vitro assays)	1171	943
Remission	6	8
Active disease	10	2

disease and a negative response to 2,4-dinitrochlorobenzene (DNCB), lymphocytes from 4 of the 10 patients tested responded normally to PHA (*19, 20*). Similar results have been observed in other series (*10, 21*).

In animals, preservation of a normal response to PHA is thought to indicate retention of the T-cell pool of lymphocytes, which includes those cells involved in cellular immunity (*22*). In man, the evidence, though less complete, suggests the same conclusion (*23*). The finding that some patients with Hodgkin's disease preserve a normal response to PHA even when skin-test negative raises the possibility that defects other than depletion of the sensitive lymphocytes account for the observed impairment of cellular immunity.

To examine this possibility, we studied lymphocytes from 26 patients with Hodgkin's disease and correlated the results of antigen-induced lymphocyte proliferation and production of MIF with the results of skin test to the same antigen. The 26 patients studied could be divided into 2 groups on the basis of these immunologic tests. In group 1, consisting of 16 patients, the results of in vitro tests correlated with those of skin tests. In group 2, consisting of 10 patients, the in vitro tests showed the presence of sensitive lymphocytes while the in vivo responses to the same antigens were negative. Comparison of the clinical features in these 2 groups did not reveal any significant differences. Although there was a trend toward more patients in remission and with nodular sclerosis in group 2 than in group 1, the difference in incidence was not statistically significant.

Studies of delayed hypersensitivity in Hodgkin's disease demonstrated fluctuation of skin tests in relation to disease activity (*1*). We observed a similar finding in these studies. Two patients with initially negative tests, after treatment developed positive in vitro tests. One of these patients also converted his skin test and is in remission. The other did not convert and is in relapse. Evolution to positive skin tests in vitro and conversion of skin tests were not regularly observed. Five of the patients in group 2 have been in remission for >4 years and still have not converted their skin tests, despite positive in vitro assays.

This type of dissociation between the results obtained by in vitro assays and skin test results has been observed in significant numbers only in patients with Hodgkin's disease and, as text-figure 1 shows, never in normal subjects. Moreover, this dissociation differs from that observed in patients with sarcoidosis and mucocutaneous candidiasis, two other diseases with impaired dermal reactivity (*18, 24*). Lymphocytes from some patients with these latter diseases retain the capacity to proliferate in response to antigen but do not produce MIF. In patients with mucocutaneous candidiasis, treatment with transfer factor temporarily restores the capacity of lymphocytes to produce MIF (*25*).

In vivo and in vitro studies of cellular hypersensitivity suggest that antigen-stimulated lymphocytes respond by proliferation and elaboration of mediators that affect macrophage function, cause chemotaxis of leukocytes, are cytotoxic, and activate other lymphocytes. These mediators are thought to contribute to the biologic effects of sensitized lymphocytes in vivo possibly by acting as biologic amplifiers (*26*).

This model of cellular immunity suggests several possible explanations of our findings. The first, depletion of sensitive lymphocytes, seems unlikely for several reasons. The distribution of lymphocytes was the same in both groups. The PHA responses of lymphocytes from group 2 patients were normal. However, quantitative deficiency of the lymphocytes responsible for initiating skin reactions to these particular antigens cannot be excluded. Other possible explanations include defects in number or function of macrophages, serum factors, or altered conditions at the reaction site. Our study has shown that production of one of the mediators, MIF, by lymphocytes from patients with Hodgkin's disease, is normal. As yet information is only limited about production of other mediators by lymphocytes from these patients (*13, 14*).

The possibility of a nonlymphocyte host defect was suggested by experiments demonstrating the difficulty in transferring delayed hypersensitivity to patients with Hodgkin's disease (*27–29*). In these experiments, leukocytes from donors of known skin reactivity were given to patients with Hodgkin's disease and to normal volunteers. The dermal reactivity of the donors was transferred to the controls in 33 of 35 attempts, but the same leukocytes transferred skin reactivity to patients with Hodgkin's disease in only 2 of 39 attempts. Thus, although the cells were competent, expression of delayed hypersensitivity in patients with Hodgkin's disease was markedly inhibited.

Our findings are consistent with the existence of a nonlymphocyte defect in some patients with Hodgkin's disease, in that lymphocytes from group 2 patients functioned normally by several in vitro criteria. However, study of other lymphocyte mediators and of function of other cells such as macrophages is needed before the mechanism underlying these observations can be fully understood. Mediator assays are available and tests of macrophage function are being developed to permit such investigation.

In Hodgkin's disease, there are probably several causes of impaired cellular immunity. Studies presented elsewhere in this Symposium demonstrate defective response to DNCB in patients with early disease (*30*). Late in the disease, lymphocytopenia contributes to the defect (*31*). In vitro studies have demonstrated defective lymphocyte function at all stages (*6–10*). Our observations suggest that, in some patients with Hodgkin's disease, lymphocytes function normally, but cellular immunity is still impaired because of a nonlymphocyte host defect.

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Impaired Delayed-Hypersensitivity Responses in 154 Patients With Untreated Hodgkin's Disease [1]

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SUMMARY—Delayed-hypersensitivity responses to 2,4-dinitrochlorobenzene (DNCB) and a battery of intradermal antigens were evaluated in 154 patients with untreated Hodgkin's disease. The overall response rates to both DNCB and the intradermal antigens were diminished. Several sensitizing concentrations of DNCB were used, and there was a significantly decreased response rate when 0.5% versus 2% sensitizing concentrations of DNCB were compared, even in early stages of the disease. In a similar manner, positive responses could be elicited from patients previously negative to 5 U streptokinase/streptodornase (SKSD) by subsequent challenge with 50 U SKSD. In contrast to other reports, peripheral-blood absolute counts did not vary significantly between stages nor between DNCB responders and nonresponders. These findings suggest that the cell-mediated immune deficiency in Hodgkin's disease is not an all-or-none phenomenon and that use of higher concentrations of antigen can partially overcome it.—Natl Cancer Inst Monogr 36: 107–115, 1973.

IMPAIRED IMMUNE RESPONSES in patients with Hodgkin's disease, described by many investigators in the past, recently have caused controversy. It was reported that the antibody response remains intact in this group of patients (*1, 2*), but defects in cellular immune responses have been discovered including delayed skin-homograft rejection (*3, 4*), abnormal lymphocyte function (*1, 5–10*), and diminished delayed-hypersensitivity responses (*11–17*). Conclusions drawn from earlier reports describing a high percentage of anergic patients have been clouded by the fact that some patients had received or were receiving either chemotherapy or radiation therapy at the time of evaluation. In addition, these studies were performed before the widespread use of lymphography for detection of retroperitoneal involvement in Hodgkin's disease and thus have a high proportion of staging errors. Kaplan described a decreased response rate to the contact sensitizer 2,4-dinitrochlorobenzene (DNCB) in patients with Stage I Hodgkin's disease who had been previously treated or were undergoing radiation therapy at the time of evaluation (*18*). Animal studies provided evidence that irradiation of the regional lymph nodes after antigen deposition profoundly affects the immune mechanisms. This results in marked depression of cellular and humoral immune responses (*19*) or normal expression of one and significant depression of the other, depending on the times of irradiation (*20*).

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In 1967, Brown and associates (*1*) reported on the responses of 50 untreated patients with Hodgkin's disease to DNCB and several intradermal antigens. With a 2000 μg (2%) DNCB sensitizing dose, an overall response rate of 70% was elicited in untreated patients, while 56% of the patients reacted to at least one intradermal antigen. They concluded that the impairment of delayed hypersensitivity was much less than previously reported and the response in patients with localized disease was normal, but reactivity decreased with increasing stage of the disease. Delayed hypersensitivity was impaired most in patients with peripheral lymphocytopenia or with lymphocyte depletion in their biopsied lymph nodes.

More recently, Young and associates (*13*) published additional data on the delayed-hypersensitivity responses in 103 patients with untreated Hodgkin's disease; 49 of these patients were included in the Brown et al. report. They attempted to sensitive 90 patients with 2000 μg of DNCB, and 67% manifested a positive response on challenge. Most of the patients were also tested with a group of intradermal antigens, and a high reactivity rate was reported, especially for mumps to which 68.5% of those tested had a positive reaction.

The present study describes the results from 2 groups of patients with untreated Hodgkin's disease. These patients were evaluated with DNCB and a battery of intradermal antigens in the Division of Radiotherapy, Stanford University Medical Center. A portion of the data was previously presented in a preliminary form (*21*).

MATERIALS AND METHODS

The patient population comprised two essentially sequential subgroups of patients evaluated from mid-1969 through January 1972. The first group consisted of 94 previously untreated patients with biopsy-proved Hodgkin's disease. These patients were randomly assigned a DNCB sensitizing concentration of 0.1, 0.5, or 2% from mid-1969 through early 1971. Patients subsequently studied with DNCB received a sensitizing dose of 0.5%; 60 additional such patients were added to the study through January 1972 for a total of 154 patients.

The technique of DNCB sensitization was similar to that described by others: 0.1 ml of an acetone solution containing DNCB in the appropriate concentration was applied to the upper medial left arm in a 2.5-cm plastic ring and allowed to evaporate; the site was occluded for 5 days. Challenge was carried out by application of 0.1 ml of 0.1% and 0.01% solutions of DNCB in acetone to the volar surface of the opposite forearm, and these sites were occluded through 96 hours. Some patients received an additional challenge concentration of 0.001%; however, there were only occasional positive responses to the 0.01 and 0.001% challenge concentrations, and the data reported here are the results of the 0.1% challenge. The median sensitization to challenge interval was 14 days; approximately 90% of the patients were challenged between 10 and 21 days after sensitization. Reactions were scored in the manner described by most other investigators; i.e., erythema alone was not considered as a positive reaction, but erythema and induration, with or without vesiculation or bullae formation, were considered as a positive reaction. The responses were evaluated from 24 to 96 hours in most instances. Several patients were excluded. It was occasionally necessary to institute treatment before DNCB challenge; these patients were excluded from the study, as were a few who refused testing, those whose reactions to DNCB and intradermal antigens were not evaluated at the appropriate times, and those who had received prior treatment. In a few instances either the DNCB or intradermal antigen results alone were excluded for one of the above reasons.

Intradermal antigen reactivity was evaluated by intradermal injection of 0.1 ml of intermediate-strength, puriled protein derivative of tuberculin (0.0001 mg PPD, Merck, Sharp & Dohme), coccidioidin 1:100 (Cutter Laboratories), histoplasmin (Parke, Davis & Co.), mumps skin test antigen (Eli Lilly & Co.), candida albicans extract 1:100 (Dermatophytin "O"—Hollister-Stier Laboratories), and blastomycin (Parke, Davis & Co.). The volar surfaces of both forearms were used for testing, and ≥0.5 cm of induration was considered a positive reaction. Reactions were evaluated at 48–72 hours and in most instances from 24 to 96 hours, and a patient was scored as a responder if ≥1 intradermal tests were positive.

A subgroup of patients not tested with blastomycin did undergo intradermal testing with streptokinase/streptodornase (SKSD-Varidase, Lederle) in addition to the other intradermal antigens. The initial evaluation was carried out by intradermal injection of 0.1 ml of this preparation which contained 5 U SKSD. A number of patients negative to 5 U underwent a subsequent challenge with 50 U before treatment. A positive response to this antigen was more conservatively defined as ≥1.0 cm of induration; patients with equivocal responses were scored as negative. The SKSD results will be described separately from the other intradermal antigens to make the presentation of our data like that of others.

Extensive diagnostic workups were done (*22*), and laparotomies were performed in 147 of the 154 patients; the 7 exceptions are discussed below. The patients were staged as recommended at the Ann Arbor Symposium on Hodgkin's Disease (*23*). Absolute peripheral lymphocyte counts were obtained before cutaneous evaluation (prelaparotomy in those undergoing surgery), and only counts obtained in this Institution were used in the analyses. These were available in 141 patients, and ≥2 counts were available in 85% of this group, in which case an average was used for analysis.

The control groups for the various DNCB concentrations consisted of healthy, young-adult subjects.

RESULTS

The median age of the 154 patients was 27 years (range 7–72 years), with 71% males (109/154) and 29% females (45/154). The mean ages of the patients by stage were as follows: Stage I—31.7 years, II—28.7 years, III—27.9 years, and IV—38.0 years. Comparison of the mean ages between stages revealed a significant difference ($P<0.05$) between Stages II versus IV and III versus IV. The distribution among stages is shown in table 1 with most patients having Stage II or III disease. Seven patients did not undergo staging laparotomy, including 5 patients with marrow or other clinically evident, disseminated, extralymphatic (Stage IV) disease, one 65-year-old patient with clinical Stage IA disease, and one 64-year-old patient with clinical Stage IIA disease. The histologic subtypes according to the Rye modification of the Lukes and Butler classification (*24*) are shown in table 2. Approximately three-fourths of the patients had nodular-sclerosing Hodgkin's disease which is consistent with our previous experience. There were no significant differences between the mean ages of patients in the various histologic subtypes.

TABLE 1.—*Distribution by stage of 154 patients with untreated Hodgkin's disease*

Stage	Number and (% of total)
I	15 (9.7)
II	78 (50.6)
III	46 (29.9)
IV	15 (9.7)
Total	154

TABLE 2.—*Distribution by histologic subtype of 154 patients with untreated Hodgkin's disease*

Histologic subtype	Number and (% of total)
Lymphocyte predominance	8 (5.2)
Nodular sclerosis	115 (74.7)
Mixed cellularity	30 (19.5)
Lymphocyte depletion	1 (0.6)
Total	154

The results of cutaneous testing in the group of 94 patients randomly assigned a sensitizing concentration of DNCB are shown in table 3. Only 3 of 16 controls (19%) receiving a sensitizing concentration of 0.1% DNCB responded to the challenge; however, the response rate increased markedly with the higher sensitizing concentrations of DNCB. There was no significant difference between the response rates of 83 and 96% for the control groups receiving 0.5 or 2% sensitizing concentrations of DNCB, respectively. There were no responses in the group of patients who received the 0.1% sensitizing concentration of DNCB. The overall response rate for patients receiving either 0.5 or 2% DNCB was significantly less than that of their respective control groups, as were the response rates of patients without systemic symptoms ("A's"). A highly significant difference was also found when one compares the responses of patients with early disease, i.e., Stages I-A, II-A, or II$_E$-A, receiving 0.5 or 2% DNCB with that of their controls; in addition, the reactivity of the 0.5% group with early disease was significantly less than that of the 2% group of patients with similar disease. For a summary of selected *P* values for these and other comparisons, *see* table 5.

Table 3.—*Responses of normal control subjects and 94 patients with untreated Hodgkin's disease to intradermal antigens and to different concentrations of DNCB*

Group and stage	Intradermal antigens	DNCB sensitizing concentration 0.1%	0.5%	2.0%
Controls	—	3/16 (19)*	24/29 (83)	26/27 (96)
Hodgkin's disease				
Stages I-A, II-A, II$_E$-A	14/39 (36)	0/5	4/16 (25)	10/15 (67)
Stages II-B, II$_E$-B	6/13 (46)	—	1/5 (20)	1/8 (13)
Stages III-A, III$_S$-A, III$_{ES}$-A	2/16 (13)	0/6	2/6 (33)	2/4 (50)
Stages III$_S$-B, III$_{ES}$-B	3/11 (27)	0/3	2/8 (25)	—
Stages IV-A, IV-B	6/12 (50)	0/2	1/4 (25)	0/2
Subtotals				
All A's	16/56 (29)	0/12	6/22 (27)	12/19 (63)
All B's	15/35 (43)	0/4	4/17 (24)	1/10 (10)
Totals	31/91 (34)	0/16	10/39 (26)	13/29 (45)

* Number positive/No. tested (% positive).

In table 4, the results are tabulated for the total group considered satisfactory for evaluation, including the 87 patients who received a sensitizing concentration of 0.5% DNCB and the intradermal responses for 151 patients. The overall response rate to 0.5% DNCB remained constant, with one-quarter of the patients demonstrating a positive response on challenge. The additional patients strengthened the previous findings and when compared with the 2% group (table 3) showed that, in the subgroup of patients with Stages I-A, II-A, or II$_E$-A disease, there was a significantly decreased response rate. Patients without constitutional symptoms ("A's") compared with the 2% group also showed a significantly decreased response rate. Of interest, there was no change in response rate with stage or with the presence of constitutional symtoms. In table 5, additional *P* values are presented for these comparisons.

Table 4.—*Responses of 154 patients with untreated Hodgkin's disease to intradermal antigens and 0.5% sensitizing concentration of DNCB (total of subgroups receiving 0.5% DNCB)*

Stage	Intradermal antigens	0.5% DNCB
I-A, II-A, II$_E$-A	24/67 (36)*	9/39 (23)
I-B, II-B, II$_S$-B, II$_E$-B	9/24 (38)	3/14 (21)
III-A, III$_S$-A, III$_E$-A, III$_{ES}$-A	4/28 (14)	4/13 (31)
III-B, III$_S$-B, III$_{ES}$-B	7/17 (41)	5/14 (36)
IV-A, IV-B	7/15 (47)	1/7 (14)
Subtotals		
All A's	28/96 (29)	13/53 (25)
All B's	23/55 (42)	9/34 (26)
Totals	51/151 (34)	22/87 (25)

* Number positive/No. tested (% positive).

The intradermal antigen reactivity rate has

TABLE 5.—*Results of statistical comparisons*

Comparison	Randomized study: 94 patients 0.5% group significance	 2% group significance	Total group: 154 patients 0.5% group significance
"Overall" vs. controls	$P<<0.001$	$P<0.001$	$P<<0.001$
"All A's" vs. controls	$P<0.001$	$0.01<P<0.02$	$P<<0.001$
"All B's" vs. controls	$P<0.001$	$P<<0.001$	$P<<0.001$
"All A's" vs. "All B's"	N.S.*	$0.01<P<0.02$	N.S.*
Stages I-A, II-A, $\mathrm{II_E}$-A vs. controls	$P<0.001$	$P<0.05$	$P<<0.001$
	0.5% group vs. 2% group significance†		0.5% Total group vs. 2% group significance
"All A's"	$P<0.05$		$0.005<P<0.01$
Stages I-A, II-A, $\mathrm{II_E}$-A	$P<0.05$		$0.005<P<0.01$

* No significant difference.
† 0.5% controls vs. 2% controls, no significant difference.

remained unchanged (tables 3, 4), and responses for each antigen are shown in table 6. The responses in 151 of the total of 154 patients were considered adequate for evaluation, with 92 patients receiving all 6 antigens (blastomycin was discontinued part way through the study); 54 patients, 5 of the antigens; and 5 patients, 4 of the antigens. The overall response rate to intradermal antigens of 34% was approximately one-half that reported by Young and associates (*13*), who reported that positive intradermal responses in their patients were mainly to mumps antigen—an experience similar to that found in this study. In the present study, of the 51 patients with positive responses to ≥ 1 intradermal antigens, 40 included a positive response to mumps antigen. Young and associates reported mumps reactivity to be independent of stage, a finding confirmed in this study, since there was no difference in response rates between stages, between substages, e.g., I-A, II-A, and $\mathrm{II_E}$-A and other substages (table 6), or between patients with or without constitutional symptoms.

TABLE 6.—*Responses to individual intradermal antigens (positive/total)*

Stage	PPD	Blastomycin	Mumps	Candida	Histoplasmin	Coccidioidin
I-A, II-A, $\mathrm{II_E}$-A	1/67	0/41	21/67	2/67	3/67	2/66
I-B, II-B, $\mathrm{II_S}$-B, $\mathrm{II_E}$-B	1/24	1/13	5/24	1/24	4/24	2/23
III-A, $\mathrm{III_S}$-A, $\mathrm{III_E}$-A, $\mathrm{III_{ES}}$-A	0/28	0/16	3/28	2/28	1/28	2/25
III-B, $\mathrm{III_S}$-B, $\mathrm{III_{ES}}$-B	0/17	0/11	6/17	0/17	1/17	1/16
IV-A, IV-B	1/15	0/12	5/15	0/15	3/15	2/15
Totals	3/151	1/93	40/151	5/151	12/151	9/145

Young et al. (*13*) reported that 7 of 60 (11.7%) untreated patients did not respond to any of a battery of 6 skin tests. The validity of combining DNCB data (a new antigenic exposure) with the results of testing using intradermal antigens (a series of earlier possible antigenic exposures) is open to question. However, if one analyzes the present series in a similar manner (patients with both DNCB tests and intradermal tests considered adequate for evaluation), 47 of 87 (54%) of the 0.5% DNCB group and 7 of 28 (25%) of the

2% DNCB group were negative to all tests (excluding SKSD). A comparison of the 0.5% results with those of Young et al. yields $P<<0.001$, while the 2% data do not differ significantly from their results.

The results of SKSD skin testing in 58 patients are presented in table 7. The distribution between stages and histologic subtypes for this subgroup is similar to that of the overall group of 154 patients. A positive response to 5 U SKSD was elicited in 6 of the 58 patients (10.3%); 3 equivocal responses were scored as negative. Twenty-nine patients with negative responses to 5 U SKSD were subsequently challenged with 50 U SKSD before therapy, to which 8 (27.6%) had a positive response; 3 equivocal responses were scored as negative. The overall response rate to SKSD was markedly less than that reported in the literature, where 66–100% of controls have been reported to respond to this antigen (*11, 25, 26*). In a companion paper in this volume, Gaines and Remington reported a 93% response rate to 5 U SKSD in age- and sex-matched controls for a subpopulation of the above group (*27*). In both of the above groups, the use of an additional intradermal antigen (SKSD) revealed a group of patients positive to that antigen but negative to the usual intradermal antigens. In addition, the use of a higher concentration of antigen elicited a higher response rate in a manner analogous to that described for 2 versus 0.5% DNCB.

TABLE 7.—*Results of intradermal testing with SKSD (positive/total) in 58 patients*

Other intradermal antigens	Positive	Negative
	SKSD, 5 U	
Positive	1/58	18/58
Negative	5/58	34/58
	SKSD, 50 U	
Positive	2/29	8/29
Negative	6/29	13/29

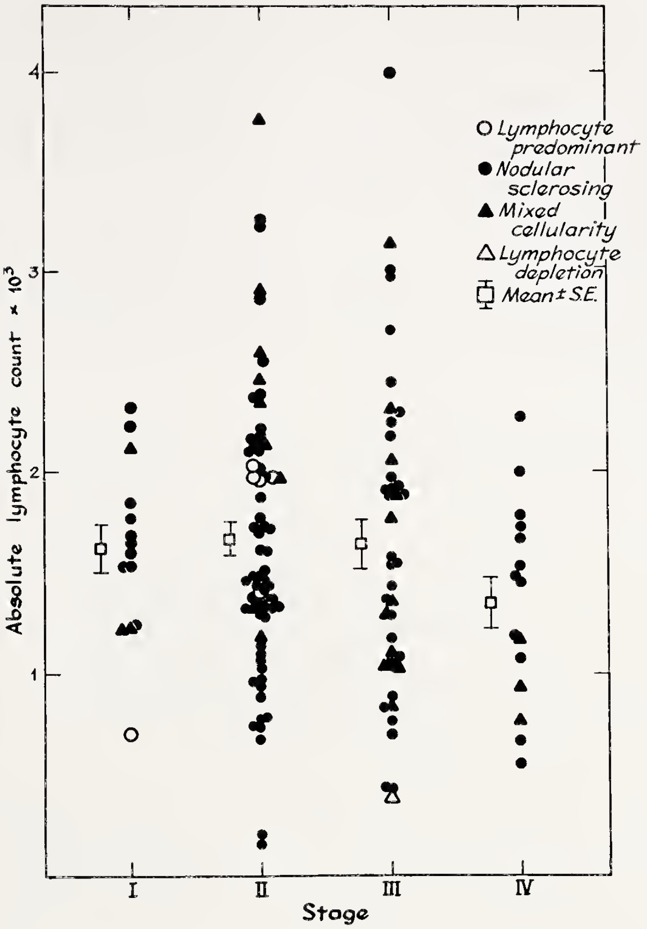
Prelaparotomy lymphocyte counts were tabulated as described above, and the values for 141 patients on whom lymphocyte counts performed in this institution were available are plotted in text-figure 1. The mean lymphocyte counts were compared by use of Student's t test (two-tail), and there was no significant difference in the mean counts between stages or for DNCB responders versus nonresponders within each stage. Similarly, there was no significant difference between the mean lymphocyte counts when comparing responders versus nonresponders to ≥ 1 intradermal antigens or to mumps antigen alone.

A more uniform subpopulation is presented in text-figure 2, where lymphocyte counts are plotted for those patients with nodular-sclerosing subtype disease who received a sensitizing concentration of 0.5% DNCB. Again, there were no significant differences between the mean lymphocyte counts; however, the numbers were small in the groups with higher stages of disease.

DISCUSSION

The findings of this study are at variance with those of Brown et al. (*1*) and Young et al. (*13*) who reported that cutaneous anergy was uncommon in their patients. These authors found a normal response rate to DNCB in Stage I patients, but significantly decreased response rates in Stages II, III, and IV. The overall DNCB response rate of 67% for all stages in the study by Young et al. does not differ significantly from the 45% overall response rate for the 2% DNCB group in the present study ($\chi^2=2.384$, $0.1<P<0.2$), though both differ significantly from their control groups. However, as shown here, tests with an 0.5% sensitizing concentration of DNCB revealed a highly significant, further decrease in the response rate in this patient population. Additional differences exist between the study of Young et al. and the present study. The response rate to the usual intradermal antigens in this report is about one-half that described by Young et al., and we were unable to confirm their finding of a significant decrease in the peripheral lymphocyte counts with increasing stage of disease. While absolute peripheral lymphocyte counts slightly decreased with increasing stage (text-fig. 1), the mean lymphocyte counts did not differ significantly between stages.

The most significant finding revealed by the present study is that patients with early disease



Text-figure 1.—Peripheral blood absolute lymphocyte count (per mm^3) related to stage of Hodgkin's disease.

(Stages I-A, II-A, and II_E-A) sensitized with 0.5% DNCB had a strikingly decreased response rate as compared with those sensitized to 2% DNCB. These results suggest that a response may be elicited in many patients by use of a sufficiently high concentration of DNCB, i.e., 2%, and conversely that use of a lower sensitizing concentration (0.5%) may unmask an immune deficiency. This is further reinforced by the results of the SKSD intradermal testing where positive responses were elicited with a 50-U challenge in patients who had been previously unresponsive to a 5-U challenge, a dose to which most normal individuals will respond.

The present study clearly reveals a significant difference in response rates between controls and patients receiving 0.5% DNCB, which is true for all stages and shows no trend with stage or the presence or absence of constitutional symptoms (table 4). This deficiency is observed in early stages in untreated patients indicating, contrary to the reports of Brown et al. (*1*) and Young et al. (*13*), that such patients do exhibit a subtle impairment of cell-mediated immune responses which is quantitative rather than all-or-none and revealed by a lower sensitizing concentration of DNCB.

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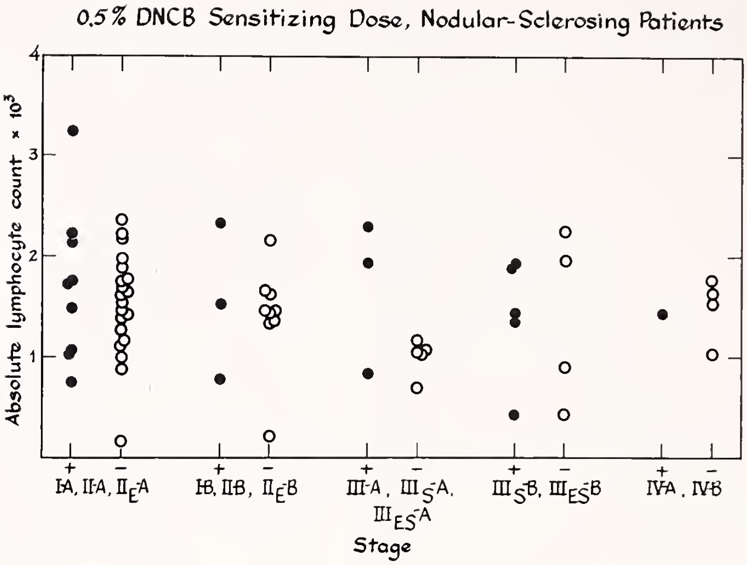

Text-figure 2.—Peripheral blood absolute lymphocyte count (per mm^3) related to response to 0.5% DNCB (+ = positive response; — = no response) and stage for patients with nodular-sclerosing subtype Hodgkin's disease.

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Deficiency of Lymphocyte Antigen Recognition in Hodgkin's Disease [1,2]

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SUMMARY—Lymphocyte transformation to specific antigens (streptokinase/streptodornase and *Toxoplasma*) was examined in 23 patients with active, untreated Hodgkin's disease and in 14 age- and sex-matched controls. Whereas lymphocyte transformation was observed in 13 of the 14 controls, it was demonstrable in only 1 of the patients with Hodgkin's disease. This defect was present early in the course of the disease as well as after treatment, when the patients were in apparent clinical remission. Plasma from Hodgkin's disease patients inhibited the transformation of lymphocytes from normal individuals, but conversely lymphocytes from patients with Hodgkin's disease, which did not transform with autologous serum, did so with normal plasma.—Natl Cancer Inst Monogr 36: 117–121, 1973.

ONE OF THE most conspicuous manifestations of the defect in cellular immunity in patients with Hodgkin's disease is infection due to opportunistic pathogens. The disseminated varicella zoster, toxoplasmosis, and cryptococcosis seen in these patients are uncommon in individuals with normal cellular immune mechanisms (*1*). Other manifestations of this defect in cellular immunity include prolonged skin homograft survival (*2*), depressed delayed skin-test reactivity (*3–5*), and decreased lymphocyte responsiveness in vitro to nonspecific mitogens such as phytohemagglutinin (PHA) (*6–9*).

Although it is generally agreed that the key cell involved in these cellular immune reactions is the lymphocyte (*10*), relatively little is known about the ability of lymphocytes from patients with staged, untreated active Hodgkin's disease to respond specifically to a previously encountered antigen.

This study evaluates antigen-specific recognition by lymphocytes of patients with Hodgkin's disease, and data are gathered to define whether disorders of cell-mediated immunity in this disease are present at the onset of clinical manifestations, or whether they develop with the progression of the disease. The data presented here must be considered preliminary.

MATERIALS AND METHODS

Subjects.—The patients with Hodgkin's disease presented consecutively for staging and evaluation of their disease at the Stanford University Hospital Radiation Therapy Clinic between June 1971 and January 1972. All had active disease, and none were treated before this

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study. Age- and sex-matched controls were healthy volunteers.

Antigens.—Toxoplasma lysate antigen, prepared as previously described (*11*), was used at 100 μg/ml. Streptokinase/streptodornase (SKSD) was supplied as "Varidase" by Lederle Co. (Pearl River, N.Y.). The commercial preparation was dissolved in distilled water, adjusted to pH 2.0 with 0.1 N HCl, dialyzed for 24 hours against 0.001 N HCl, titrated to pH 7.4 with 0.1 N NaOH, and then dialyzed for 24 hours against phosphate-buffered saline, pH 7.4. Protein was determined by the method of Folin-Ciocalteu with bovine serum albumin as a standard. Optimum levels of transformation were obtained when the preparation of SKSD was used at a protein concentration of 100 μg/ml.

Antibody determinations.—The Sabin-Feldman dye test (DT) was performed as described by Frenkel and Jacobs (*12*).

Cell preparation and culture.—For lymphocyte transformation studies, 30 ml of heparinized blood was drawn from each individual before the skin test, and the leukocytes were prepared for culturing in 20% autologous plasma by a modified procedure previously described (*13*). Cells at 1×10^6/ml were cultured in either tissue culture medium-199 (TC-199, Grand Island Biological Co., Berkeley, Calif.) without antigen, or medium containing either *Toxoplasma* or SKSD antigen. After 6 days' incubation in an atmosphere of 5% CO_2 and 95% air, 2.0 μCi of tritium-labeled thymidine (^{3}H-TDR) (specific activity = 6.0 Ci/mmole; Schwarz/Mann, Orangeburg, N.Y.) was added to each tube; 18 hours later the cells were washed with 0.15 M NaCl, extracted with cold 5% trichloroacetic acid in absolute methanol, and prepared for scintillation counting.

The degree of lymphocyte transformation or level of ^{3}H-TDR uptake is indicated by the ratio A/C, where A equals the counts per minute (cpm) of lymphocyte cultures incubated with antigen, and C the cpm of lymphocytes incubated without antigen.

RESULTS

Toxoplasma Serology and SKSD Skin Tests

The 23 patients with Hodgkin's disease ranged in age from 18–76 years, with a mean of 33 years (table 1). Eighteen (78%) were male. Of the 20 patients staged, 1 was Stage I, 11 Stage II (6 were II-A and 5 were II-B), 5 Stage III, and 3 Stage IV. There were 14 controls ranging in age from 21–58 years, with a mean of 32 years; 10 (71%) were male.

TABLE 1.—*Data in 23 patients with Hodgkin's disease*

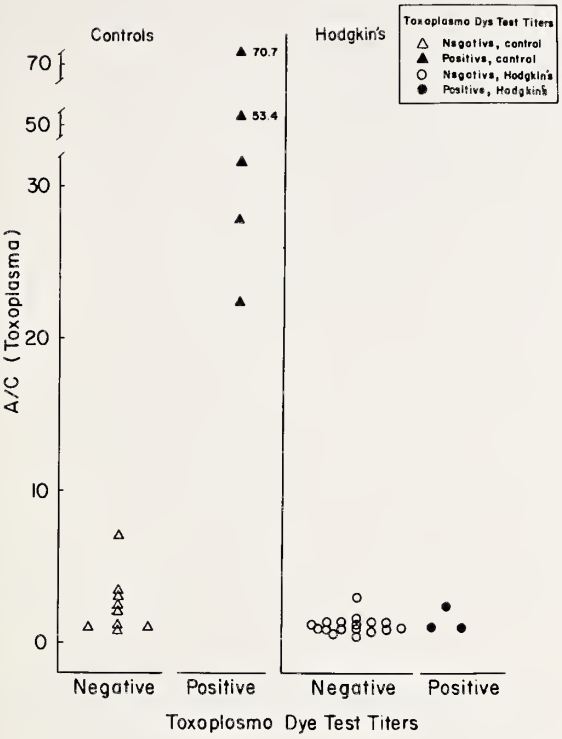
Subjects (No.)	$\bar{X}$ Age (range)	+ SKSD skin test (5μ)	+ *Toxoplasma* Dye-test titer (≥1:8)
Hodgkin's disease (23)	33.0 (18–76)	2 (9%)	3 (13%)
Controls (14)	31.8 (21–58)	13 (93%)	5 (36%)

Skin sensitivity to 5 U SKSD was present in 9% of the Hodgkin's disease patients and in 93% of the controls (*see* table 1; text-fig. 3). Serologic evidence of prior infection with *Toxoplasma* was present in 13% of the Hodgkin's disease patients and in 35% of the controls (table 1).

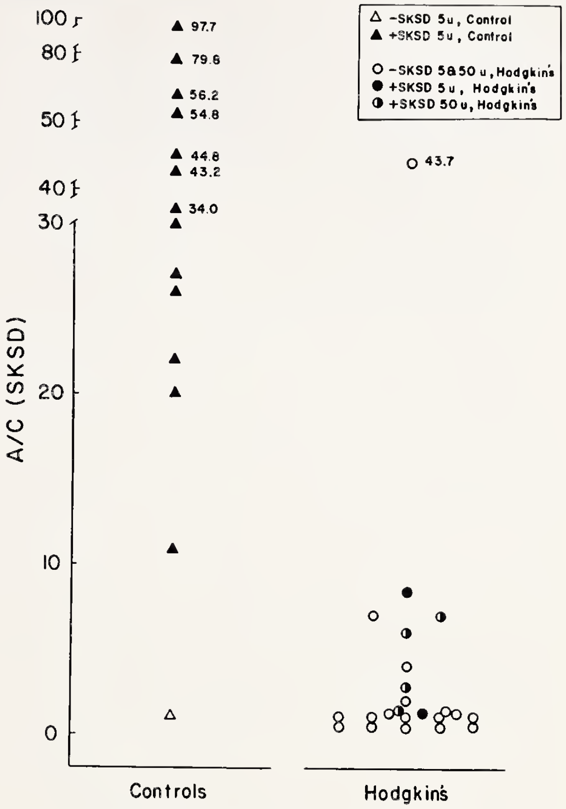
Lymphocyte Transformation Studies

The A/C ratios with *Toxoplasma* antigen are shown in text-figure 1. The ratio in control individuals who had no serologic evidence of prior infection with *Toxoplasma* ranged from 0.5 to 7.0, indicating little or no ^{3}H-TDR uptake in the presence of antigen. This contrasts with the A/C ratios of 22–71 observed in control subjects with positive *Toxoplasma* serology. In the Hodgkin's disease patients, neither those individuals with negative serology nor the 3 patients with positive serology had significant lymphocyte transformation.

The A/C ratios in response to SKSD antigen are shown in text-figure 2. Thirteen of the four-



Text-figure 1.—Lymphocyte transformation to *Toxoplasma* antigen in Hodgkin's disease.



Text-figure 2.—Lymphocyte transformation to SKSD in Hodgkin's disease.

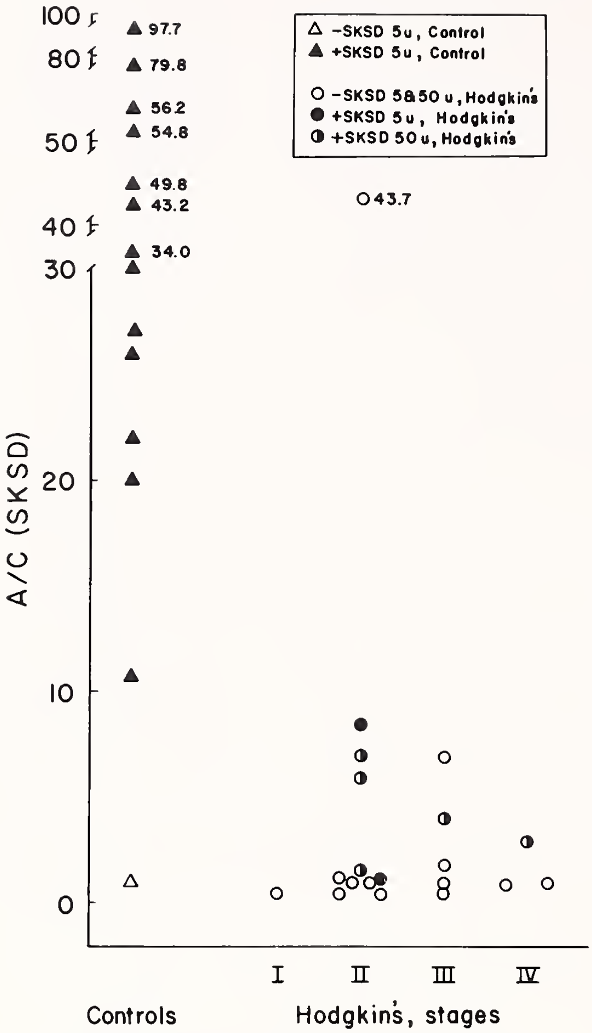
teen controls had a positive skin test to 5 U SKSD, and their *A/C* ratios ranged from 11 to 98. The lymphocytes of the single skin-test-negative control subject did not transform. In the group of patients with Hodgkin's disease, only 2 had positive SKSD skin tests when tested with 5 U. Four who were negative to this dosage reacted when retested with 50 U. Lymphocytes of 22 of the 23 patients with active Hodgkin's disease did not transform in vitro to SKSD. The response of lymphocytes to this antigen in relationship to staging in 20 of the patients is seen in text-figure 3. The 1 patient whose lymphocytes responded did so at a high level. The patient was in Stage II. None of 6 patients with Hodgkin's disease who were retested at 2½ months post therapy, when they were in apparent clinical remission, demonstrated significant lymphocyte transformation to SKSD.

Plasma Experiments

To determine whether factors in the plasma may be at least partly responsible for the inhibition of lymphocyte transformation in patients with Hodgkin's disease, studies were carried out with autologous and homologous plasma in 2 patients with Hodgkin's disease (before therapy) and in 3 controls (table 2). Plasma from the patients with Hodgkin's disease remarkably blocked the ability of lymphocytes from normal individuals to transform to SKSD. When lymphocytes from Hodgkin's patients were cultured with plasma from normal individuals, they transformed, but not as well as those of controls when cultured in their own plasma. Culturing lymphocytes from controls in plasma from other normal controls did not significantly alter the level of ^{3}H-TDR uptake.

DISCUSSION

Although preliminary, these data suggest that lymphocytes from individuals with Hodgkin's disease do not transform to an antigenic stimu-



TEXT-FIGURE 3.—Lymphocyte transformation to SKSD as related to stage of Hodgkin's disease.

lus which produces a response in almost 100% of normal individuals. This defect is present early in the course of the disease and may be present in patients in apparent remission. These data contrast with those obtained with skin testing (*3, 4*) and in in vitro lymphocyte transformation studies (*7–9*), in which both cutaneous anergy and defects in lymphocyte transformation were related to the activity and stage of the disease.

An explanation for the difference in our lymphocyte transformation results from those of others may be that we were using specific antigens (*Toxoplasma* and SKSD) as opposed to nonspecific mitogens such as PHA. In a recent report, Han and Sokal (*10*) questioned the use of PHA in evaluating lymphocyte responsiveness as related to specific immunity. They suggested that PHA constitutes a very strong stimulus to the lymphocyte and fails to detect depression of immunologic function which is demonstrable by other techniques.

TABLE 2.—*Effects of plasma on lymphocyte transformation in patients with Hodgkin's disease and in normal controls*

Subjects		
Cells	Plasma	*A/C*
Patient 1	Autologous	0.5
Patient 1	Control A	6.9
Control A	Autologous	20.7
Control A	Patient 1	2.1
Patient 2	Autologous	1.6
Patient 2	Control B	15.3
Patient 2	Control C	34.2
Control B	Autologous	110.1
Control B	Patient 2	14.4
Control C	Autologous	92.9
Control C	Patient 2	9.9
Control B	Control C	76.5
Control C	Control B	104.9

Toxoplasma antigen was selected for the present study because serologic evidence of persistent infection with this organism is present in 30–50% of normal adults in the United States (*15*). Since resistance to *Toxoplasma* appears to be cell mediated (*11, 16, 17*), we considered this an ideal system to evaluate the cellular immunologic status to latent intracellular parasitism in compromised patients. The reason for the comparatively lower incidence of positive serologic titers among patients with Hodgkin's disease (13%) as compared with controls (35%) is unclear and is being studied in our laboratory. SKSD was employed because it can be used for both in vivo and in vitro testing, and previous studies have shown that between 70 and 100% of normal individuals have a positive skin test to this antigen (*5, 18*).

We observed that plasma from untreated patients with Hodgkin's disease inhibited transformation of lymphocytes from normal individuals. Lymphocytes from patients with Hodgkin's disease which did not transform, or which did so poorly in the presence of autologous serum, did transform in the presence of normal plasma. Trubowitz et al. (*19*) also demonstrated an inhibitory effect of plasma from patients with Hodgkin's disease on transformation of lymphocytes from normal individuals as well as a slight stimulatory effect of normal plasma on the ability of lymphocytes from patients with Hodgkin's disease to transform. These results contrast with those reported by Hersh and Oppenheim (*6*) who found only slight stimulatory effect of normal plasma on cells from patients with Hodgkin's disease and no effect (in 2 of 3 cases) of plasma from these patients on normal cells. In contrast to our patients, most of those in the latter investigations had been treated before study. In addition, the studies were performed with a nonspecific mitogen (PHA).

Even though we have not yet performed lymphocyte-transformation determinations on sufficient numbers of individuals with Stage I disease, our data suggest that impairment of delayed hypersensitivity may be present at the onset of the disease. Whether this defect is an etiologic factor rather than a consequence of Hodgkin's disease remains controversial (*20*). We were not interested in defining whether these patients can respond to any given number of antigens—a question asked and answered by many other investigators (*3–5*). We desired solely to define whether there is a significant difference in specific cellular recognition between normal individuals and individuals with early Hodgkin's disease, and there is. To further resolve the relation of absence of lymphocyte recognition to the basic disease process, it will be necessary to perform determinations at later intervals after therapy to define what occurs to in vitro parameters of delayed hypersensitivity in patients in remission, as apart from the effects of therapy itself.

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Invited Discussion: Kinetic Approach to the Study of Cell-Mediated Immunity in Hodgkin's Disease [1,2]

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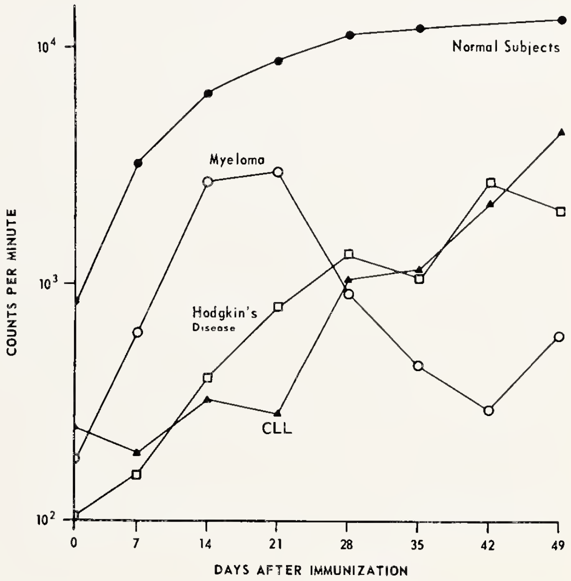
IN OPENING this discussion, I would like to propose a slightly different approach to the evaluation of the immune responsiveness of patients with Hodgkin's disease. Specifically, I refer to a kinetic approach involving immunization with an individual antigen and subsequent kinetic follow-up of both the cell-mediated and humoral immune responses. Some years ago we studied patients with Hodgkin's disease and compared their immune responses to keyhole limpet hemocyanin (KLHC) with the responses to this antigen by patients with chronic lymphocytic leukemia (CLL), multiple myeloma, and normal subjects. We believe that a kinetic approach of this type reveals patterns of abnormal responsiveness which may have some bearing on the etiology of these diseases.

We immunized small groups of patients with KLHC and serially followed their antibody responses, their in vitro lymphocyte blastogenic responses, and their delayed-hypersensitivity skin-test responses weekly after immunization. Studies of the in vitro lymphocyte blastogenic responses to stimulation with KLHC (measured by tritiated thymidine incorporation) revealed that all groups of patients had a deficient response but that the pattern of response in each disease was distinct and unique (text-fig. 1).



TEXT-FIGURE 1.—Primary immune response to KLHC—blastogenesis.

We also studied the antibody responses of the patients with the various lymphoid malignancies and compared these responses with those of normal controls (text-fig. 2). The responses of patients with solid tumors were similar to those of a control group. The responses of patients with multiple myeloma rose fairly promptly but were not maintained. Those of patients with Hodgkin's disease were somewhat delayed, then rose moderately, and then fell. Surprisingly, the responses of patients with CLL were very delayed, but the antibody titers eventually attained normal levels.

In contrast, our studies of delayed-hypersensitivity skin-test reactivity to KLHC revealed that only the patients with Hodgkin's disease had a markedly depressed ability to develop delayed hypersensitivity to KLHC, though the

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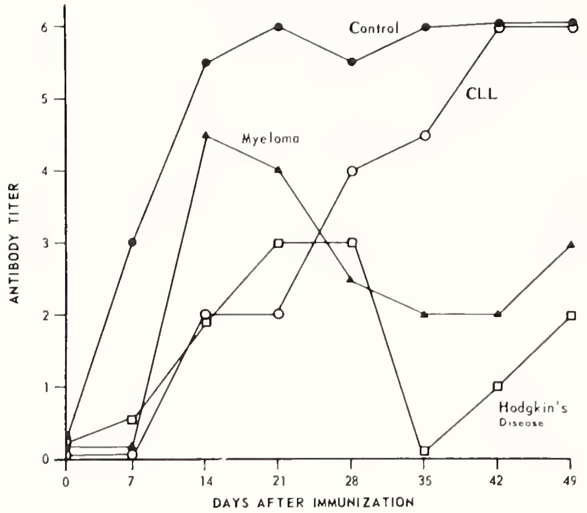
[2] Supported by Public Health Service Contract PH4368949 from the collaborative research program, Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases.

Table 1.—*Delayed-hypersensitivity responses of control and study groups to challenge with KLHC*

Diagnosis	Number tested/No. positive (%)		Reaction diameter	
			Median	Range
Normal	14/14	(100)	10.3	5–30
Localized solid tumor	9/9	(100)	15.7	5–25
Metastatic solid tumor	11/9	(85)	8.0	0–15.5
Hodgkin's disease	13/6	(46)	4.8	0–20
Multiple myeloma	22/18	(82)	6.5	0–15.5
CLL	13/12	(92)	8.6	0–13.0



Text-figure 2.—Antibody production in patients with selected malignancies and in control subjects.

other groups were somewhat suppressed compared to our controls (table 1).

These data illustrate the points I would like to make in this discussion. First, through such kinetic studies, unique patterns of immunologic deficiency can be identified in the various lymphoid malignancies. Since there appear to be unique patterns of deficiency, this may imply unique etiologies of these different diseases. Second, although the major emphasis in Hodgkin's disease has been placed on patients' cell-mediated immune defect, there also appears to be a significant defect in their ability to produce a humoral antibody response. This suggests either a defect in B-cell as well as T-cell function or a T-cell defect involving both those T-cells involved with cell-mediated immunity and those involved with helper function in the antibody response.

SUMMARY OF INFORMAL DISCUSSION ON CELL-MEDIATED IMMUNITY

R. A. Good commented that, with respect to the total numbers of lymphocytes, he had recently had some very telling experiences studying patients with the DiGeorge syndrome, a syndrome in which children are born without a thymus, and therefore do not have any T-cells, but have normal numbers of lymphocytes. If one looks at those lymphocytes with techniques which allow one to quantify the different classes of the lymphocytes, one will find that instead of about 20–30% of the circulating cells being B-lymphocytes, that fraction is sometimes >90%. So the void created by the absence of lymphocyte population can be filled by another. It would be very interesting in Hodgkin's disease to do a precise quantitation of T-cells and of the different classes of B-cells with this new methodology.

H. S. Kaplan underscored one point that emerges from the papers given by Doctors Eltringham and Churchill. The earlier studies by Brown and associates and later by Young and associates from the National Cancer Institute yielded evidence which seemed to exclude the possibility that the immune-deficiency state was a primary event or at least concurrent in time with primary events in Hodgkin's disease. However, the randomized study which Doctor Eltringham presented rather puts the shoe on the other foot and suggests that there is an immunologic deficiency state, albeit a very subtle one that is easily masked, which is present even in patients with extremely early, sometimes unifocal Hodgkin's disease. Thus, we cannot eliminate the possibility that this abnormality is a primary event or one associated with the earliest manifestations of the disease. Accordingly, it must once again be taken into consideration in the context of our discussions of the etiology of Hodgkin's disease.

Good, commenting on the paper by Doctors Gaines and Remington, pointed out a discrepancy between the responses in Hodgkin's disease to nonspecific mitogens and those to the specific antigens they studied. In some studies, the nonspecific mitogen responses of Hodgkin's patients seem to be normal, i.e., a measure of the general T-cell population. He suggested the need to look at quantitative methods for measuring the T-cells rather than at a technique that involves a concentration of the T-cells, because with so many of the cells capable of responding to a nonspecific mitogen, one might just get "noise."

Genetic Factors in Hodgkin's Disease: Association With a Disease-Susceptibility Locus (*DSA*) in the HL-A Region [1,2]

W. F. Bodmer,[3] ***Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford, England***

SUMMARY—The statistical problems of studies on associations between diseases and genetic polymorphisms such as the HL-A system are reviewed. Special emphasis is placed on the bias involved in the assessment of the true significance of an extreme association picked from a number of comparisons. Serological difficulties connected with HL-A typing of diseased patients could also complicate detection of HL-A and disease associations. An overall assessment of published data on the HL-A antigen distribution in Hodgkin's disease patients and controls seems to confirm the association with the "4c" related set of antigens, HL-A5, W5, W15, and W18. This association is interpreted in terms of the effects of a disease susceptibility locus, *DSA*, in the HL-A region.—Natl Cancer Inst Monogr 36: 127–134, 1973.

AGING, accidents and anxiety, genes, and infection are among the major underlying causes of disease. In our modern industrialized society, we have learned to control most of the common infections that were until quite recently the major scourges of mankind. Control of the other four factors, however, remains much more elusive. As a result, medical research tends to concentrate more and more on diseases such as Hodgkin's which are not obviously due to an infectious agent. Epidemiologic and other data suggest a viral etiology for Hodgkin's disease in common with other tumors, as extensively discussed elsewhere in this Symposium. This paper reviews the evidence for an interaction between genes and infection as a major factor in the causation of Hodgkin's disease. The gene or genes involved appear to be closely linked to those that determine the antigens of the major human histocompatibility system, HL-A.

Traditionally the major clue to a genetic basis for a disease has come from family studies and from the comparative analysis of incidence among relatives and unrelated controls. Even if a disease does not show the familiar Mendelian patterns indicative of a single gene determination, it may nevertheless have a predominantly genetic basis due to the joint action of two or more genes. Such a multifactorial inheritance is generally indicated by a much higher incidence among relatives of affected individuals, especially identical twins, than in the population at large [for review, *see (1)*, chapter 9]. Hodgkin's disease apparently does not show high concordance rates in identical twins or a marked familial incidence (2). The data of Razis et al. (2) suggest that the incidence of Hodgkin's disease may increase approximately threefold among first-degree relatives of affected individuals as compared with controls. Such an increase, however, could easily be accounted for by transmission of an infectious agent, which has a lo probability of giving rise to observable diseas among family members. At most, therefore, thes

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data would suggest that, if one or more genes are involved in susceptibility to Hodgkin's disease, the environment must still be a predominant factor in its causation.

An alternative approach to the search for genetic factors in such a situation is to look for specific genetic markers that might show an association with the disease. Based on the assumption that the red blood cell groups and other similar polymorphisms must be maintained in the population by some form of balancing selection, Ford (*3*) urged a search for associations between blood groups and specific diseases. The idea that there might be associations between the ABO blood groups and disease must have been in Landsteiner's mind when he discovered them in 1900. The first convincing association between blood type A and cancer of the stomach was, however, only reported in 1953 (*4*). Many other significant associations involving the ABO and other red blood cell groups have been reported since, e.g., (*5*). In no case, however, is the mechanism underlying the association properly understood and few, if any, of the associations seem significant with respect to natural selection, which was the original motivation behind their discovery. There is one report on the distribution of ABO blood groups in Hodgkin's disease (*6*) which suggests a slight deficiency of type A and an accompanying excess of type B associated with the disease, but, to my knowledge, this has not been followed up or confirmed. Such studies are, in general, only worth doing if there is some specific reason or hypothesis for associating a particular genetic polymorphism with any given disease.

A rationale for the existence of some associations between histocompatibility-antigen systems and certain diseases was provided by two important lines of investigation. First, Lilly and others (*7, 8*) showed that susceptibility to the Gross virus-induced form of murine leukemia is controlled by two or more genes, at least one of which is closely associated with the *H-2* locus, the major histocompatibility system of the mouse. Second, McDevitt and Benacerraf (*9*) and others showed that the mouse and the guinea pig have genes which control the immune response to certain antigens closely linked to the major histocompatibility locus. This led to the suggestion that histocompatibility-linked genes affecting susceptibility to tumor and other viruses act through their effects on the immune system (*10*). Following the stimulus of these studies, many investigators have begun to search in man for associations between the antigens of the HL-A system and certain diseases. The first significant association was reported by Amiel (*11*). He showed that one of the antigens (then called 4c) of the HL-A system occurred almost twice as often in patients with Hodgkin's disease than in his control population. There have been many further reports on the distribution of HL-A antigens in Hodgkin's disease patients, and the evaluation and interpretation of these results are the main concerns of this paper.

HL-A SYSTEM AND DISEASE ASSOCIATION

It is now generally accepted that the HL-A antigens can be separated into 2 major series, LA and 4, each of which behaves as if its constituent antigens were controlled by a set of alleles at a single locus (*12*). The HL-A system shows a remarkable degree of genetic variation, most phenotypes occurring with frequencies of well under 1%. At present, at least 13 antigens in the LA series and 16 antigens in the 4 series have been described. Pedigree data show that the *LA* and *4* loci are closely linked with a recombination fraction of about 0.8% (*13*). A recombination fraction of this order of magnitude, however, probably encompasses up to 2,000 genes, among which may be included analogues of McDevitt's immune-response loci and loci controlling the mixed-lymphocyte culture (MLC) response and graft rejection (*14, 15*).

The large number of antigens of the HL-A system emphasizes a major statistical pitfall that must be avoided in assessing disease antigen associations, i.e., overestimating the significance of an association because it is an extreme one picked out of many possibilities. This is a well-known statistical problem, all too often overlooked in such studies. The problem arises when a number of comparisons are being made and the most extreme one is picked out because of interest. One cannot use the normal statistical tests on this selected association, because these tests do not allow for such selection. Thus, if we are comparing the frequency of 20 antigens in patients with Hodgkin's disease and their controls, we should expect, on the average, that even if none of the antigens really is associated with Hodgkin's disease, nevertheless, by the nature of the significance test, 1 antigen, on the average, will differ significantly at a 5% level when assessed by the usual chi-square test. A rough correction for taking into account this problem is to multiply the normal significance level by the number of comparisons to get an approximation of the true significance level for the selected comparison. Thus, for example, with 20 comparisons, a true significance level of 5% would require a significance level of about 0.25%, with the usual statistical tests. The best way to be sure of any suggested association is to do an independent follow-up survey and confirm the initially suggested association. The association being looked for is then stipulated a priori, and so the statistical problem of picking out the extreme deviate does not arise.

There are 2 other very important methodologic problems in the search for such associations. The first is the proper choice of the control populations. Clearly any stratification of the population associated with differences in the HL-A antigen frequencies could lead to spurious associations. Most obviously, since the antigen frequencies vary between races, the racial composition of patients and control groups must be matched. Since there are no known associations between HL-A antigens and age or sex, matching for these variables may not be important. In general, the smaller the potential difference between disease group and control, the larger the sample studied must be to obtain significance and the more careful the choice of the control. Sibs have been suggested as ideal controls, but this may often not be true. Sib controls may be biased due to problems of ascertainment, may limit the range of differences that can be compared because of the restrictions implied by genetic segregation within the families being studied, and may be biased with respect to their environmental contacts. Usually, attempts are made to obtain controls from the same population pool from which the Hodgkin's disease patients were drawn, e.g., patients admitted to the same hospital with other diseases.

The second methodologic problem is the proper classification of the diseased patients. If the disease has a heterogeneous etiology, it is quite likely that only some forms of the disease and not others will show a given association. Not taking account of the disease heterogeneity may, therefore, dilute the significance of an association but is unlikely to lead to a spurious association. Partial association may, of course, itself help to define different categories of the disease.

There are at least 2 serologic complications that could, in principle, lead to confusing or spurious associations. Antigens specific to the disease may exist, e.g., tumor- or virus-associated antigens, which are detected by antibodies in some, but not all, of the sera used for HL-A typing. Such antibodies will presumably not show up in the normal course of screening HL-A sera for their specificity. Sera containing such antibodies would, however, be expected to react quite anomalously in diseased patients as compared to controls, unless the disease antigen was itself associated, or cross-reactive, with one of the HL-A antigens. An antigen analogous to such a postulated disease-associated antigen, which is specific for some of the in vitro established lymphoid human cell lines, was described in (*16*).

The second serologic complexity could arise if the peripheral blood lymphocytes, routinely used for HL-A typing, from Hodgkin's disease patients do not react normally in the standard cytotoxic, complement-dependent assay. Bertrams et al. (*17*) described a case of Hodgkin's disease in which there appeared to be transient "loss" of HL-A antigens after ^{60}Co-irradiation treatment. Another more subtle possibility is that the cells from diseased patients become more susceptible to cross-reacting antibodies. Cross-reaction among antigens of the HL-A system is very common and seems to occur in more or less defined patterns (*18*). Thus, it may be that sera reacting weakly on normal cells, if at all, with a given cross-reacting specificity, would react more strongly with cells from a Hodgkin's disease patient carrying this specificity. This could lead to spurious antigen assignments and thus confuse associations with antigens, and especially to differences between studies due to the particular sera used. Some anomalies recently observed in the HL-A typing of lymphoid cell lines are perhaps explainable in this way (*16, 19, 20*).

Both of the above potential serologic complications can, in principle, be overcome by doing family studies to establish unequivocally the *HL-A* genotypes of the patients and controls. This is not always possible and certainly entails much more work.

Finally, one major genetic factor could greatly complicate observed associations between HL-A antigens and diseases. This arises if the association is, as discussed in the introduction, not with the HL-A antigens themselves but with the effects of a closely linked locus. In this case, the HL-A antigens are merely acting as markers for the presence of a given allele at the linked locus. The association may then be considerably disturbed by the occurrence of crossing-over between the disease locus and the *HL-A* loci. Associations will then be found only with given HL-A antigens to the extent that these tend to be found in the population being studied in coupling, namely, on the same haplotype, with the relevant disease locus alleles, that is, if disease and *HL-A* loci are in linkage disequilibrium. Thus, for example, most pairs of antigens, one from the LA series and the other from the 4 series, do not show significant population association. Only certain pairs, such as HL-A1 and 8 and HL-A3 and 7 in Caucasian populations, are significantly associated due to linkage disequilibrium. Such associations may, moreover, vary from one population to another. The more closely linked the loci, the higher the average level of significant linkage disequilibrium (*21, 22*). This problem so far has not arisen in the mouse studies, because these are done with a limited number of inbred lines that can be crossed, intercrossed, and backcrossed at will to obtain direct linkage information.

HL-A AND HODGKIN'S DISEASE

The list of obstacles that must be overcome to establish a convincing case for an association between one or more HL-A antigens and a disease seems formidable. The apparent success with Hodgkin's disease is perhaps due to the intense interest in the subject, which has already produced 10 independent reports covering a total of 682 patients and to the probable relatively large magnitude of the association.

Amiel's original report (*11*) of an association between Hodgkin's disease and the antigen 4c was based on 41 patients and gave a chi-square test result of 5.06 for the significance of the association, corresponding to a significance level of about 2.5%. Taking into account the fact that he was then typing for 5 antigens and this was the one extreme result, the true significance level should have been only 12.5%, which might not be thought to merit serious attention. Subsequent studies, however, confirmed and extended this observation to antigens different from, but associated with, the antigen 4c studied by Amiel (*11*).

The antigen 4c, first described by Bodmer and Payne (*23*), using an agglutination assay, was probably the same as the antigen now known as HL-A5. Subsequent studies on this antigen by use of the lymphocytotoxicity assay, which has since been the basis for most HL-A typing, were for technical reasons based on a serum called Rafter (or 1027). Rafter was known to include the original 4c antigen but also to react with other specificities. Absorption of serum Rafter showed that it was not a mixture and so suggested that its antibody cross-reacted with HL-A5 and other, as yet, undefined specificities (*24, 25*). It is now known (*12*) that the antibody in Rafter cross-reacts with 3 specificities: HL-A5, W5, and W18. A number of sera have now been described which clearly identify HL-A5 without any involvement with W5 and W18. Other sera have also been defined which cross-react only with HL-A5 and W5 but not with W18, or which react only with W5 or only with W18. These latter seem, however, less reliable than the others. In this way, it is now possible to classify separately for HL-A5, W5, and W18, though these 3 antigens form a well-defined cross-reactive cluster. One further antigen, W15, is also known to belong to this cross-reacting group of antigens (*18*). W15 is of special interest, since it has recently been associated with systemic lupus erythematosis (SLE) (*26, 27*).

After Amiel's original observation (*11*), 2 independent studies, one by Forbes and Morris (*28*) and the other by Zervas et al. (*29*), claimed to confirm the association of 4c with Hodgkin's disease. However, while Zervas et al. (*29*) maintained that the association was with HL-A5, Forbes and Morris (*28*) implicated W5. Seven later published studies on HL-A and Hodgkin's disease variously implicated W18 and W15, as well as HL-A5 and W5, though in no case does more than 1 of these 4 antigens show a significant association. In the 9 studies published after Amiel's (*11*), W18, W5, and W15 were each significant in 2 and HL-A5, in 3 studies (*see* table 1 for detailed references). If we consider these 9 studies as follow-up studies on Amiel's report (*11*), then in assessing their overall significance, we no longer need to take into account the bias due to selecting extreme results. The pooling of data on the 4 cross-reacting antigens associated with 4c can also perhaps be considered as an a priori consideration for these studies. The question is then whether the combined data from these studies on all 4 antigens agree in showing a significant effect. A simple way to look at this question is to ask whether there are significantly more high chi-square values (say >3.8, the 5% value for 1 df)) involving the 4c antigens than there are for all the other HL-A antigens. Table 1 lists the 155 comparisons made in these 9 studies.

TABLE 1.—*Comparison of chi-square values for association between Hodgkin's disease and 4c cross-reacting antigens versus all other HL-A antigens**

	Number of chi-square values		
	<3.8	>3.8	
4c antigens (HL-A5, W5, W18, and W15)	19	9	28
Other HL-A antigens	116	11	127
	135	20	155

*The chi-square value for this 2×2 table is 9.25 ($0.5\%>P>0.1\%$). Data are taken from the following nine studies: Zervas et al. (*29*), Morris and Forbes (*30*), Coukell et al. (*31*), Thorsby et al. (*32*), van Rood and van Leeuwen (*33*), Kissmeyer-Nielsen et al. (*34*), Bertrams et al. (*35*), Falk and Osoba (*36*), and Jeannet and Magnin (*37*).

Of 28 comparisons involving the 4c antigens, 9 gave chi-square values >3.8, while of 127 comparisons involving other HL-A antigens, only 11 gave chi-square values >3.8. This difference was significant at a P level of between 0.5 and 0.1%. The fact that even 11 chi-square values >3.8 were nearly twice the expected number ($\frac{1}{20} \times 127 = 6.35$), a difference which is itself significant at the 5% level, suggests that other antigens are significantly associated with Hodgkin's disease. Two of the studies (*34, 36*) in fact showed significant associations with both the antigens HL-A1 and HL-A8. The close association between these 2 antigens in the Caucasian population makes it likely that, if one is significantly elevated in frequency, the other will also be. In this context, it is also of interest that HL-A8 showed a significant increase in the SLE patients described by Grumet et al. (*26*). These associations do not, however, confirm previous suggestions, and so are subject to the correction for bias discussed above. Further data will be needed to assess whether HL-A8, and perhaps HL-A1, may really be associated with Hodgkin's disease to the same extent as are the 4c-related antigens.

It is hoped that many of these questions will be resolved by the cooperative study on Hodgkin's disease which forms a part of the next International Histocompatibility Testing Workshop. About 20 independent groups of Hodgkin's disease patients and properly chosen controls will be typed with a common, highly selected panel of HL-A sera. The diagnosis of Hodgkin's disease will furthermore be assessed by an independent panel of expert pathologists, and data will be collected on the histologic type, staging, treatment, and other parameters of the disease.

DISCUSSION

There can now be little doubt of the overall significance of the association between Hodgkin's disease and the 4c cross-reacting set of HL-A antigens. Two features of the data are at first glance puzzling: Not all Hodgkin's disease patients have the antigens in question, and the association is not simply with one of the

HL-A antigens, but with a related cluster of antigens.

The fact that not all Hodgkin's disease patients have the implicated HL-A antigens is in line with the point emphasized by McDevitt and Bodmer (*10*) that histocompatibility-linked genes may be only one of several genes affecting susceptibility to such diseases. Moreover, the effect of the genes may not be clearly all-or-none, but may simply be to significantly increase or decrease the probability of getting the disease, e.g., through an effect on the individual's susceptibility to a virus.

The second feature of the data, i.e., association with a set of antigens rather than with just 1 antigen, must be interpreted in the context of postulated mechanisms underlying such an association. Snell (*38*) suggested 2 mechanisms which could associate the histocompatibility antigens themselves with susceptibility to virus infection and so perhaps to certain cancers: 1) when there is a similarity between a histocompatibility antigen and a viral antigenic determinant; and 2) when the histocompatibility antigen provides, or interacts with, viral receptor sites on the cell surface. In either case it could be argued that these relations would still hold, to some extent, for a set of cross-reacting antigens. The third major mechanism to explain histocompatibility antigen-disease associations is through the effects of a closely linked immune response locus, as already discussed. This latter hypothesis is certainly the most appealing, and as we shall now discuss, fits in well with the observed data and current knowledge on the HL-A system.

As was emphasized earlier, if the association is with a disease-susceptibility locus in the HL-A region, which can be called *DSA,* then because of recombination it is not expected that the association would necessarily be with a single given antigen. The association would only be with 1 antigen if the relevant allele at the *DSA* locus responsible for increased susceptibility to Hodgkin's disease, say *DSA1,* occurred only in, or predominantly in, a haplotype with the genetic determinant for this particular antigen. Such extreme linkage disequilibrium would only be expected if the *DSA* locus was very close to, for example, the *4* locus of the HL-A system, or if the *DSA1* allele had arisen comparatively recently. The fact that the observed association is with a family of antigens suggests that the postulated allele, *DSA1,* is found predominantly in haplotypes with the genetic determinants for the antigens HL-A5, W5, W15, and W18. The extent to which *DSA1* occurs in combination with other haplotypes may be a factor in explaining why not all Hodgkin's disease patients have one of the 4c-related antigens. In other words, the association of the 4c-related antigens with Hodgkin's disease would be explained by their being in relative linkage disequilibrium with the allele *DSA1* at the closely linked *DSA* locus, whereas other antigens are, in general, not so associated with *DSA1.* The association between HL-A8 and HL-A1 and Hodgkin's disease, if confirmed, could either be explained by linkage disequilibrium involving *DSA1* and the *HL-A8* and *HL-A1* alleles or by a linkage disequilibrium involving another allele at the *DSA1* locus (or even at another closely linked *DSB* locus). This would also confer increased susceptibility to Hodgkin's disease. The variation between studies with respect to which antigen shows a significant association could be explained simply by chance variations in the frequencies of the *DSA1*-containing haplotype.

Heterogeneity in the nature of the disease could, of course, be another factor causing differences between studies. The frequency, for example, of the 4 histologic types certainly seems to differ significantly from one center to another; Forbes and Morris (*28*) and Falk and Osoba (*36*) have suggested that different forms of the disease show different associations. Conceivably these differences could be associated with different alleles at the *DSA* locus or with different *DS* loci, not all of which are necessarily closely linked to *HL-A*. However, much more data are needed to assess the significance of associations with subdivisions of Hodgkin's disease; it may well turn out that the major subdivisions are all varying stages or manifestations of the same disease associated with just the one postulated *DSA1* allele.

Bodmer (*15*) suggested that related cross-reacting antigens such as the 4c set have been derived from each other, or from precursor alleles, by recombination. This could account for the tendency for an allele, such as *DSA1,* at a closely linked locus to be in disequilibrium with a number of related antigens. The fact that the disequilibrium is predominantly with alleles of the *4* locus suggests the postulated *DSA* locus is closer to the *4* than to the *LA* locus of the HL-A system. This is consistent with the observation made by Grumet et al. (*26*) in their SLE patients that the association with a particular *4* locus antigen (in this case W15) was not explicable by an association with a haplotype including a particular *LA* locus allele. The *4* locus is also the end of the HL-A system, which seems to be predominantly associated with the MLC response (*39, 40*) and so with the MLC locus (*14*). It is of interest in this context that the *Ir-1* locus of McDevitt and co-workers (*9, 10*) is nearer the K than the D end of the *H-2* region and that this is the end that may be predominantly associated with the MLC response and skin-graft rejection (*41, 42*) and thus the end which appears to correspond to the *4* locus of the *HL-A* system.

Further work on Hodgkin's disease and other diseases will be needed to establish how much polymorphism there might be for the *DSA* and other similar loci. It is also important to see whether this genetic variation is significant for any of the more common, and therefore evolutionarily more important, infectious diseases.

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Invited Discussion: Genetic Factors in Immune Responsiveness[1]

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IN THE mouse, as in man, there are 2 closely linked loci which control the major cell surface antigens responsible for graft rejection. As W. F. Bodmer has pointed out earlier, in man these are called the *4* locus and the *LA* locus. In the mouse, the analogous loci are called *H-2K* and *H-2D,* and these 2 loci are separated by approximately 0.5 recombination map units.

In recent years, it has become apparent that the immune response to a large number of specific antigens and specific antigenic determinants in the mouse, the rat, and the guinea pig is under an autosomal dominant genetic control. These genes, designated Immune-response genes or *Ir* genes, share a number of characteristics (*1*). They are all dominant, they are antigen specific, and they appear to be expressed primarily in thymus-derived, antigen-reactive cells called T-cells, although the evidence for this is not yet complete. The information presently available suggests that we are dealing with genes which control the recognition of carrier determinants on antigens and result in the stimulation of the helper function subserved by thymus-derived lymphocytes in inducing bone marrow-derived lymphocytes to proliferate, differentiate, and produce specific antibody. In addition, in the mouse, where genetic mapping techniques are available, a number of *Ir* genes have been mapped in close association with the *H-2K* locus of the *H-2* complex. The *Ir* genes map in the left center part of the *H-2* complex, and recent evidence has shown that the genes controlling the antigens that elicit the mixed-lymphocyte reaction also map in the same position.

As has been noted earlier, there are a number of mouse models in which disease susceptibility is associated with a particular *H-2* genotype. Among these are Gross virus-induced leukemia, Friend virus leukemia, and susceptibility to lymphocytic choriomeningitis virus. In each of these animal models, the pattern of inheritance of *H-2*-related susceptibility fits with the concept that the association with *H-2* is mediated via an *Ir* gene. *Ir* genes are dominant and the pattern of inheritance of susceptibility or resistance in each of these models is compatible with this interpretation.

As you have heard, there appear to be a number of associations between particular HL-A histocompatibility types in man and particular disease states. The pattern of inheritance has not yet been sorted out for any of them, and studies are currently under way to determine whether the association between HL-A type and a particular disease entity reflects the effect of HL-A-linked *Ir* genes of the type already described in the mouse, the rat, and the guinea pig. The association with Hodgkin's disease does not on first examination appear to be explicable on the basis of an *Ir* gene because the association appears to be one between a particular set of cross-reacting HL-A antigens and *susceptibility* to Hodgkin's disease (*2*). However, Falk and her colleagues in Toronto have recently published preliminary evidence which suggests that the association between these particular HL-A antigens and Hodgkin's disease is only apparent in the long-term survivors, and that there is no particular predilection for any HL-A type in recently diagnosed patients. The implication of this finding would be that the long-term survivors tend to have a particular HL-A antigenic type and that this HL-A type may be associated with resistance to the progression of Hodgkin's disease. Clearly a great deal more work will have to be done before the nature of these associations can be worked out in detail. It is most important that family studies be carried out to determine the accuracy of HL-A typing and to rule out the possibility that we are detecting disease-associated antigens. In addition, associations between HL-A and susceptibility to a disease such as Hodgkin's disease raise the possibility that the particular histocompatibility antigens may cross-react with exogenous pathogens such as viruses and lead to susceptibility via cross-tolerance rather than via the mechanism of an *Ir* gene. A third possibility is that particular HL-A antigens may function as viral receptors, and this again would tend to make susceptibility dominant, rather than resistance.

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As Dr. Bodmer has pointed out, if the associations between particular disease entities and HL-A antigens are the effect of linked *Ir* genes, the association might be quite imperfect because of crossing-over in a randombred population. This would suggest that we should develop techniques for detecting *Ir* genes in man, and for determining the phenotype, and if possible the genotype, of *Ir* genes in man. These studies are currently under way and may be of considerable importance because of recent findings that the number of diseases which have been associated with particular HL-A antigens is expanding. In addition to Hodgkin's disease and systemic lupus erythematosus, there is now impressive evidence that acute lymphocytic leukemia is associated with a particular HL-A type; that gluten-sensitive enteropathy has a very high incidence of HL-A8 and that psoriasis is also associated with the inheritance of particular HL-A antigens in family studies. Almost all of the HL-A antigens associated with specific disease entities are at the *4* locus, which is analogous to the situation in the mouse where the *Ir* genes and the gene controlling resistance to Gross virus-induced leukemia are associated with the *H-2K* locus. While most of the disease entities that have been associated with specific HL-A antigens have an immunologic component, either in their pathogenesis or in host resistance, a great deal more study will be necessary to establish that *Ir* genes exist in man and that the association of particular HL-A types with particular disease entities is mediated, at least in some of the instances, via the mechanism of an HL-A linked *Ir* gene.

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SUMMARY OF INFORMAL DISCUSSION ON GENETIC MARKERS

R. A. Good commented that one of his colleagues has shown 2 kinds of susceptibility to Friend virus. One confers relative resistance and the other absolute resistance. Relative resistance can be wiped out by antilymphocyte serum, whereas absolute resistance cannot. The relative resistance does not seem to be associated with an immune resistance gene. The absolute resistance form does. Perhaps there can be another set of relationships with this sort of genetic determinant.

C. G. Schmidt called attention to the studies of his group in Essen, West Germany, who have found an increased frequency of W-18 in patients with Hodgkin's disease.[1]

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Hodgkin's Disease-Associated Antigens: Studies on Segregation and Specificities [1,2]

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SUMMARY—Heterologous antisera derived from immunization with Hodgkin's tumor infiltrate and absorbed with normal splenic tissue was used to demonstrate 2 antigens associated with Hodgkin's disease. The antigens were also found in several autoimmune diseases and fetal tissue. Preliminary studies indicated that one of the antigens is associated with lymphocytes from the tumor infiltrate. The results of these studies were reviewed in light of the pathogenesis of Hodgkin's disease and possible future applications.—Natl Cancer Inst Monogr 36: 139–145, 1973.

ABSORBED heterologous antisera can be used to identify tumor-associated antigens in a variety of clinical tumors (*1–5*). Tumors analyzed for such antigens by both immunofluorescence and gel diffusion are usually composed primarily of cells clearly identified as neoplastic cells, e.g., malignant melanoma, sarcoma, and colonic carcinoma. In contrast, the cellular components of Hodgkin's disease often are predominantly host cells involved in a reaction which defies precise definition (*6*). The present research describes 2 antigens identified in Hodgkin's disease infiltrates, embryonic tissue, and other pathologic processes involving immune reactions. Our concept of the role of these antigens in the pathogenesis of Hodgkin's disease and future possible application of these antigens are discussed.

MATERIALS AND METHODS

Splenectomy was performed during clinical staging of Hodgkin's disease and through this means we obtained surgically removed spleens. Spleens and lymph nodes received at the surgical pathology desk during the normal hospital routine were also accepted. Only surgical specimens were used, and all samples were sequentially numbered regardless of the pathologic description. Cell suspensions were prepared with a sterile mortar and pestle from all tissues received. For immunization, 2 g tissue in 3 ml saline was prepared at room temperature and then counted with a hemocytometer and stored in 4×10^8 cell samples at –20° C. For immunoelectrophoresis, tumor, other clinical disease tissue, normal controls, and fetal tissue antigens were prepared with 1 g tissue/5 ml saline and frozen at —20° C. The remaining tissue was stored in petri dishes at —80° C. The spleens removed from patients with known Hodgkin's

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disease afforded us 4 different histologic situations: *Hodgkin's disease-positive spleen,* bears a tumor infiltrate; *Hodgkin's disease-negative spleen,* no evidence of disease histologically; *Hodgkin's disease-suspicious spleen,* abnormal histology but not diagnostic of Hodgkin's disease, and *"normal region,"* the tissue adjacent to a tumor infiltrate.

Immunization and antiserum production.—Rabbits were immunized with tumor cells from a fresh surgical specimen or from a previously frozen sample. The cells (4×10^8) from an individual specimen were mixed with an equal volume of Freund's adjuvant (Difco), and a total volume of 2–3 ml was injected intradermally. Two weeks later, 4×10^8 cells in 2–3 ml Tyrode's solution were injected intraperitoneally, followed by similar injections on days 21 and 28. One week after the last intraperitoneal booster injection, intracardiac bleeding was carried out. The defibrinated blood was refrigerated overnight, centrifuged at 2,000 rpm for 15 minutes at 4° C, heat-inactivated at 56° C for 30 minutes, and stored in 1 ml samples at —20° C.

Absorption of antiserum.—For preparation of cell suspensions used for absorption of antiserum, frozen surgical specimens of normal splenic tissue and splenic tissue from the normal region of a Hodgkin's disease-positive spleen were suspended in Tyrode's solution. Absorption was carried out by incubation of 1 ml antiserum for 15 minutes at room temperature with 1×10^8 freshly prepared cells from the normal spleens or the normal region of a Hodgkin's disease-positive spleen. The material was then centrifuged at 900 rpm for 10 minutes at 4° C, and the supernatant removed. When further absorptions were desired, the process was repeated, as described in (*7–9*). Absorption was carried out with each antiserum until no precipitin bands were obtained when 2 normal spleen extracts were tested by immunoelectrophoresis.

Immunoelectrophoresis.—One μl of previously frozen homogenized antigen was dispensed twice in the sample well of an agarose film-cassett system (Analytical Chemists, Inc.). The second μliter was added to the sample well only after the first had dispersed. The buffer (ethylenediaminetetraacetic acid, barbital, pH 8.6) and the electrophoretic system were supplied by Analytical Chemists, Inc. Eight antigens migrated in different tracts in film charged with 15 v/cm; the film was removed after 35 minutes. Twenty μl of antiserum to be tested was loaded in the trough and the process repeated after complete absorption of the first sample. The agarose film was then placed in a humidified incubator at 25° C for 48 hours. After immunodiffusion, the plates were equilibrated for 90 minutes in 0.9% saline, fixed for 10 minutes in 0.1% tannic acid, stained for 10 minutes in 0.2% 8-anilino-1-naphtholenesulfonic acid in 5% acetic acid, and rinsed for 1 minute in 5% acetic acid. The plates were photographed with Polaroid 200 film, with indirect lighting, and scored for the presence and position of precipitin bands. Two normal spleen antigens were placed in every plate as negative controls and two tumors known to yield precipitin bands were used as positive controls. The remaining 4 sample wells were used for experimental antigen extracts.

RESULTS

All immunoelectrophoretic plates had 2 normal splenic antigens that were negative controls and therefore did not result in precipitin lines. Similarly, 2 Hodgkin's tumor extracts were used as positive controls that uniformly yielded characteristic precipitin bands. In the remaining 4 antigen wells, resultant precipitin bands were scored and yielded, when present, either S (slow-migrating) or F (fast-migrating) antigens, or both. S antigen was closer to the sample well and cathode, whereas F antigen was more distal and nearer the anode (fig. 1).

Splenic Tumor Antigens

Of 23 Hodgkin's tumor infiltrates, 22 reacted with an F band and 9 concomitant with S bands (table 1). The 1 antigenically negative spleen was reviewed by Dr. Ronald Dorfman, who diagnosed nodular lymphoma rather than Hodgkin's disease. There was no instance of S antigen alone in a splenic tumor infiltrate.

TABLE 1.—*Immunoelectrophoresis of Hodgkin's disease (HD) in splenic tumor extracts**

Antigen(s)	Number of patients	Histologic type of HD
F	13	3 LD 5 MC 5 NS
SF	9	3 LD 3 MC 3 NS
No reaction	1	1 MC†
Total positive reactions	22/23	

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* Controls consisted of 2 negative normal splenic extracts and 2 positive HD splenic extracts. Tumor antisera were absorbed with normal spleen cells. Abbreviations: MC, mixed-cellularity; NS, nodular sclerosis; LD, lymphocyte-depleted HD; S, slow-migrating antigen; and F, fast-migrating antigen.

† Due to the negative antigenic result, the slides of this specimen were sent for consultation and review to Dr. Ronald Dorfman. His diagnosis was nodular lymphoma rather than HD.

TABLE 2.—*Immunoelectrophoresis of other splenic extracts from Hodgkin's disease (HD) patients**

Histology	Antigens	Number of positives / Number of patients
Negative	4 F 1 S 2 SF	7/14
Normal region	1 F	1/4
Suspicious	2 F 1 SF	3/4

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* Abbreviations: *Normal region* refers to an uninvolved area of a spleen that contained Hodgkin's disease infiltrate. *Suspicious* refers to spleens that had changes suggesting HD but Reed-Sternberg cells could not be identified. *Negative spleens* are histologically without evidence of disease but removed from a patient with known HD. S, slow-migrating antigen; and F, fast-migrating antigen.

Antigens From Spleens of Hodgkin's Disease Patients

From 14 spleens without tumor infiltrate removed from patients with known Hodgkin's disease (negative spleens), there were 7 antigenically positive results, with 6 of the 7 having F antigen and 3 having S antigen (table 2). Of 4 extracts taken from apparently normal regions adjacent to tumor infiltrate, 1 was antigenically positive and, in this instance, was adjacent to the largest tumor mass. In those spleens histologically suspicious, lacking only confirmatory Reed-Sternberg cells, 3 of 4 spleens were F-antigen positive, with one instance of a concomitant S antigen.

Antigens of Other Diseases

Assessments of other disease states yielded 8 of 27 positive spleens (table 3). Three of these spleens were involved in immune reactions. Lymphoid hyperplasia in 2 was associated with idiopathic thrombocytopenic purpura and chronic glomerulonephritis, a third spleen with histiocytic and plasma cell predominance was associated with aplastic anemia. Two other spleens containing granulomas were associated with sarcoid and leukemia and were positive for F antigen. In each of these instances F antigen was identified but not S. S antigen was identified in a ruptured spleen. The explanation of this finding remains unclear.

Fetal Antigens

Analysis of fetal antigen extracts of the liver, spleen, and thymus, ranging from 8 to 40 weeks, and 1 neonate are shown in table 4. Of 8 fetal livers, 8 were positive, with 7 having F antigen and only the most immature (8 weeks) being positive for S antigen. Of 4 splenic extracts, 3 yielded positive results for F antigen only. Analysis of thymus gland extracts demonstrated 1 positive neonatal gland with S and F antigen and 6 fetal glands, all negative. Antigen extracts prepared from fetal gut (0/2), lung (0/3), muscle (0/1), placenta (0/1), and amniotic fluid (0/1) were nonreactive.

Antisera Derived From Different Histologic Types of Hodgkin's Disease

Three different antisera were also compared for reactivity against a composite group of representative antigens. Each antiserum was derived by immunization with a different histologic form of the disease, i.e., lymphocyte-depleted, mixed-cellularity, and nodular sclerosis Hodgkin's disease. These antisera after appropriate

TABLE 3.—*Immunoelectrophoresis of spleens from other clinical states**

Clinical indication	Histology	Precipitin bands
Peptic ulcer	Normal	0
Pancreatitis	Normal	0
Dx. lap.	Lymphosarcoma	0
Myelofibrosis	Myeloid metaplasia	0
Portal hypertension	Chronic congestion	0
HD-infectious mononucleosis	Infectious mononucleosis	0
Cancer of esophagus	Normal	0
Teratocarcinoma of testis	Normal	0
ITP	Follicular hyperplasia	0
Portal hypertension	Fibro congestion	0
ITP	Normal	0
Diverticulitis of colon	Normal	0
Hiatal hernia	Normal	0
ITP	Normal	0
ITP	Lymphoid hyperplasia	0
Renal cell carcinoma	Normal	0
Cancer of esophagus	Normal	0
Hiatal hernia	Normal	0
CGN	Normal	0
Splenic rupture	Normal	SF
Peptic ulcer	Heterotopic bone formation	F
Aplastic anemia	Plasma cells, histiocytes	F
ITP	Hyperplastic follicles	F
Hiatal hernia	Normal	F
CGN	Lymphoid hyperplasia	F
Anemia, leukemia	Lipid granuloma	F
Sarcoid	Non-caseating granuloma	8/27

* Abbreviations: 0, no precipitin band; dx. lap., diagnostic laparotomy; HD-infectious mononucleosis, patient had infectious mononucleosis and later developed Hodgkin's disease; ITP, idiopathic thrombocytopenic purpura; CGN, chronic glomerulonephritis; F, fast-migrating antigen, and S, slow-migrating antigen.

TABLE 4.—*Immunoelectrophoresis of fetal antigens**

Gestation age (wk)	Thymus	Liver	Spleen
8	—	S	—
10	0	F	0
10	0	F	F
12	—	F	—
15	0	F	—
19	0	F	—
32	0	F	F
40	0	F	F
40†	FS	—	—
	1/7	8/8	3/4

* Abbreviations: F, fast-migrating antigen; S, slow-migrating antigen; —, no tissue sample available; and 0, no precipitin band.

† Died 2 weeks after gestation.

absorption were then tested with the negative normal and positive tumor controls, as well as lymph node tumor, other splenic tumor, and fetal tissue. The results are still preliminary (table 5).

TABLE 5.—*Results of immunoelectrophoresis with antisera derived from different histologic types of Hodgkin's disease (HD)*

Antigens	Heterologous antisera		
	LD	MC	NS
Normal spleen	0	0	0
Normal spleen	0	0	0
Splenic tumor	0	SF	SF
Splenic tumor	F	SF	SF
Splenic tumor	F	SF	SF
Nodal tumor	0	SF	SF
Neonatal thymus	F	SF	F
Fetal liver	0	SF	F

* Abbreviations: LD, lymphocyte-depleted; MC, mixed-cellularity, and NS, nodular sclerosis histologic type of HD. S, slow-migrating antigen; and F, fast-migrating antigen.

Lymph Node Antigens

Analysis of lymph-node tumor infiltrates demonstrated 5 of 6 positive for antigen; all had S antigens and 1 had concomitant F antigen. However, there is no instance as yet of F antigen alone (table 6). In contrast, lymph nodes from other diseases have not as yet yielded positive results (table 7).

TABLE 6.—*Immunoelectrophoresis of Hodgkin's disease (HD) lymph node tumors**

Antigen(s)	Number of patients	Histologic type of HD
S	4	1 MC 2 LD 1†
SF	1	1 MC
0	1	1 NS
Total positive reactions	5/6	

* Abbreviations: 0, no precipitin band; F, fast-migrating antigen; S, slow-migrating antigen; MC, mixed-cellularity; LD, lymphocyte-depleted; and NS, nodular sclerosis HD.

† Type of HD not defined.

TABLE 7.—*Immunoelectrophoresis of lymph nodes—other diseases**

Clinical indication	Lymph node histology	Antigens
Uterine fibroids	Normal	0
Lung carcinoma	Squamous cell carcinoma	0
Cancer of esophagus	Adenocarcinoma	0
Lung lesion	Sarcoid	0
Lung lesion	Sarcoid	0
		0/5

* 0, no precipitin bands.

DISCUSSION

In an initial report, we reviewed the results of immunofluorescence of splenic tumor infiltrates in 3 Hodgkin's disease patients and demonstrated common antigenicity (*7*). Subsequently, we reported 7 patients with similar results with immunofluorescence (*8*). In addition, gel-diffusion techniques revealed common antigenicity among patients with tumor infiltrates; while in each instance normal splenic extract controls were negative. To further rule out normal splenic antigens, an absorbed antiserum derived from normal spleen was shown to lack similar reactivity to tumor infiltrates as tumor antisera did (*8*). An absorbed antiserum derived by immunization against human γ-globulin was also shown to lack significant tumor reactivity (*9*). Finally, preliminary immunoelectrophoresis data indicated 2 possible antigens in some tumor infiltrates (*10*). Our present report deals with immunoelectrophoretic analysis of these 2 antigens which, based on the migrating pattern in immunoelectrophoresis, we designated as S (slow-migrating) and F (fast-migrating) antigens.

From the present data, F antigen is predominant in splenic tumor infiltrates (22/23) but relatively lacking in lymph node tumor (1/6). The negative splenic tumor infiltrate, which did not react antigenically, was reviewed by Dr. Ronald Dorfman whose diagnosis differed from our pathologist. We had hoped to obtain a cellular description which might explain lack of antigenicity; however, Dr. Dorfman's diagnosis was nodular lymphoma rather than Hodgkin's disease. F antigen was readily identifiable in fetal liver (7/8), fetal spleen (3/4), and in some pathologic situations where immunity is an important factor (idiopathic thrombocytopenic purpura, chronic glomerulonephritis, and aplastic anemia). It was also present in a sarcoid and leukemic spleen with granulomas. The data suggest the relationship of F antigen to fetal hematopoiesis and adult immune reactivity; however, this must be thoroughly investigated before any conclusion concerning the cellular source of the antigen can be reached. F antigen is probably not related to carcinoembryonic antigen, since it was not found in fetal gut.

More recent and preliminary work with Dr. J. Colgan in our laboratory has yielded a method of segregation of lymphocytes, lymphoblasts, and reticulum cells from the remaining cellular milieu by discontinuous bovine serum-albumin gradient centrifugation. Subsequent immunoelectrophoresis of the various cellular layers revealed F antigen to be associated with lymphocytes, lymphoblasts, and reticulum cells.

In contrast, S antigen was predominant in

Hodgkin's lymph-node extracts (5/6) and when present in the spleen was associated with F antigen. The presence of S antigen in the most premature fetal liver (8 weeks' gestation) and neonatal thymus may ultimately allow us to determine its role in fetal development as well as in Hodgkin's disease. S antigen has not been identified in the other fetal organs studied (gut, lung, and muscle). It will be of considerable interest to determine the association of S antigen with specific cellular components of Hodgkin's disease and also to determine its concentration in lymph node extracts; both of these aspects are presently under study.

In summary, during the pathogenesis of Hodgkin's disease, 2 antigens are elaborated, S and F. The F antigen is also found in diseases associated with immune processes and in some fetal organs concerned with hematopoiesis. The restriction of S and F antigens to fetal primitive hematopoietic organs and a neonatal lymphopoietic organ may have significance in relationship to hypothesized autoimmunity in Hodgkin's disease (*9–13*).

The hypothesis that Hodgkin's disease is a complex of autoimmunity and malignancy is consistent with many features of the disease [*see* (*13*) for discussion]. It would not be surprising if lymphocytic activity in this ill-defined cellular reaction were a source of these antigens. If we are to understand the pathologic process to modify it, then these antigens may be of considerable use as clues to the pathogenesis of the disease.

Possible areas of clinical application include detection of early disease, reactivation of disease, and patient monitoring during and after therapy. With the success presently available with the standard treatment modalities, radiation and chemotherapy, more precise definition of distribution of the disease would provide an even greater opportunity for clinical success. Although use of heteroimmune sera is considered by some as a possible treatment modality, either with or without radioactive tagging, we would caution against this and prefer extensive investigation and definition of the antigenic specificities before such consideration. The observation of "blocking antibody" and immunologic enhancement, as described in many malignancies by the Hellströms (*14*), must be taken into account as well as the present-day success with the available treatment modalities. New information about the nature of the cellular infiltrate and the interactions of host cells in the pathogenesis of Hodgkin's disease may now become available through the use of these antigens.

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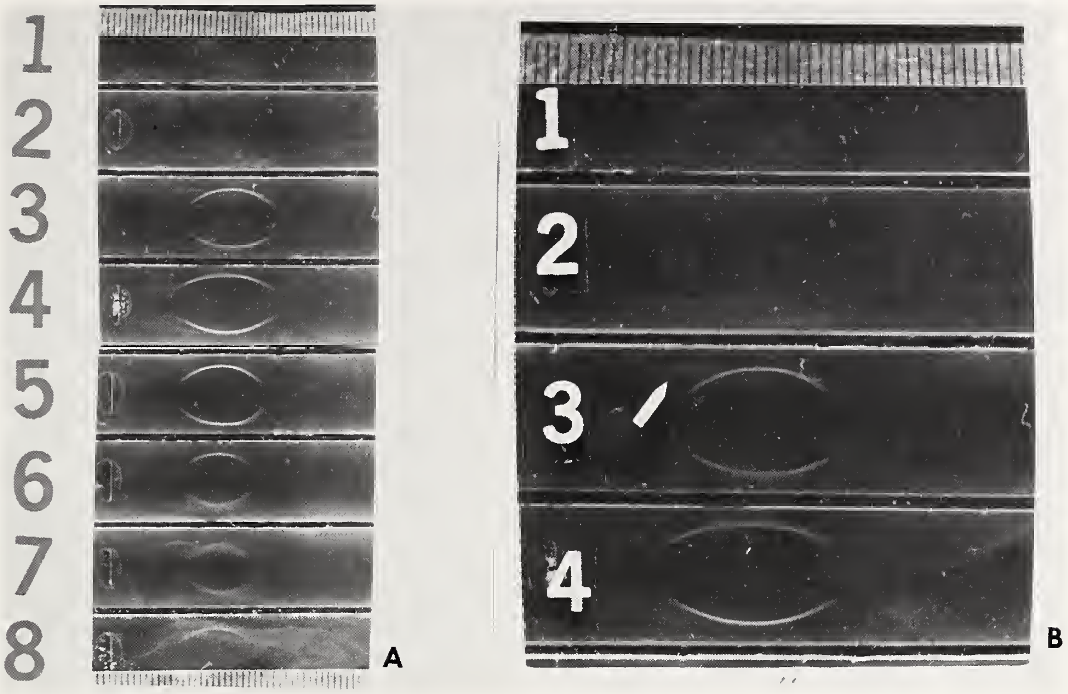

FIGURE 1.—A) Results of immunoelectrophoresis of 8 antigen samples. Antigen wells 1 and 2 contain normal spleen antigens and no precipitin bands are present. Remaining 6 samples (3 through 8) are antigen extracts from Hodgkin's tumor infiltrates and are all positive with distinct precipitin bands. B) The first 4 antigens consist of 2 normal negative controls (no precipitin bands), and 2 posiive tumor controls with both S, slow-migrating (*arrow*), and F, fast-migrating, antigens.

Detection of Ferritin as a Circulating Tumor-Associated Antigen in Hodgkin's Disease [1,2]

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SUMMARY—Ferritin, initially detected as a tumor-associated antigen in low pH extractions of Hodgkin's disease tumor homogenates, was found, by counterelectrophoretic techniques, to circulate in the sera of patients with a variety of diseases, including Hodgkin's disease, but not in normal sera. Elevated serum ferritin was present in 68% of patients with Stages III and IV nodular-sclerosing Hodgkin's disease, and in 32% of patients with Stages I and II disease. With immunofluorescence techniques, ferritin was found as a cytoplasmic protein in the stroma of normal tissues, and within spindle cells and macrophages in Hodgkin's disease tumor nodules. Ferritin could not be detected in tumor lymphocytes, atypical mononuclear cells, or Reed-Sternberg cells. Ferritin was also found in the serum of 28% of patients with non-Hodgkin's lymphomas, 60% of patients with hepatitis, and in a few patients with metastatic tumors. Although nonspecific, ferritin levels detectable by CEP are abnormal and, as such, could be useful in assessing Hodgkin's disease activity.—Natl Cancer Inst Monogr 36: 147–157, 1973.

TUMOR-ASSOCIATED antigens have been reported for many human tumors including colonic carcinoma (*1*), neuroblastoma (*2*), melanoma (*3*), Burkitt's lymphoma (*4*), and hepatoma (*5*). In a few instances serologic detection of these antigens has made it possible to study their potential in assessing disease activity. The antisera for colonic carcinoembryonic antigen (CEA) and hepatoma (α-fetoprotein) are available for this purpose, though CEA has been reported in other tumors beside colonic carcinoma (*6*) and α-fetoprotein is not present in all cases of hepatoma (*7*).

Although not usually considered a tumor-associated antigen, large quantities of ferritin can be isolated from the splenic tumor nodules of Hodgkin's disease. Ferritin, which occurs intracellularly and is nondetectable in normal sera except by radioimmunoassay (*8*), has been described in the serum of patients with hypertension (*9*), shock (*9*), hepatitis (*10*), and various malignancies (*10, 11*). Hyperferritinemia of Hodgkin's disease has been reported by Reissman and Dietrich in patients with liver involvement and by Aungst in patients with extensive disease (*10, 11*). This report describes the tissue localization of ferritin in Hodgkin's disease and its serologic presence in 108 untreated patients undergoing staging laparotomy.

MATERIALS AND METHODS

Tissue preparation.—Spleens were obtained from patients with Hodgkin's disease at staging

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laparotomy, and those with extensive tumor used to prepare a tissue homogenate. The tumor nodules were excised and put through a Harvard tissue press. An equal volume of phosphate-buffered saline (PBS) (0.15M, pH 7) was added and the tissue homogenized in a Waring blendor for 10 minutes at 4° C. The homogenate was centrifuged at 5000 rpm for 20 minutes at 4° C and the supernatant decanted. In initial studies the sediment was resuspended in PBS and the centrifugation and resuspension were repeated until the supernatant was clear. Ten volumes of citrate buffer (pH 3.2, .02M) were then added to the sediment and stirred at 37° C for 2 hours. After centrifugation, the supernatant was decanted (the pH adjusted to 7.5 with 0.1N NaOH) and concentrated by pressure dialysis to a protein concentration of approximately 10 mg/ml. After identification of the tumor-associated antigen as ferritin, crystalline ferritin was prepared from Hodgkin's disease splenic tumor nodules. To the minced tumor were added 3 volumes of distilled water. After homogenization, the slurry was heated to 65° C for 10 minutes and centrifuged for 20 minutes at 5000 rpm. The supernatant was then brought to pH 4.2 by the addition of acetic acid and the precipitated protein removed by centrifugation. The supernatant was adjusted to pH 7 by the addition of 1N NaOH and precipitated with 35% ammonium sulfate. The precipitate was dissolved in distilled water and crystallized twice with $CdSO_4$ according to the method of Granick (*12*). Protein was determined by the method of Lowry et al. (*13*), with horse ferritin as a standard.

Disc electrophoresis and immunogen preparation.—Proteins were separated by disc electrophoresis at pH 8.5 in 7.5% polyacrylamide gels with glycine reservoir buffer (*14*). For immunization, approximately 100 μg of pH 3.2 extracted protein was electrophoresed on each of 12 acrylamide gels. With the use of 1 acrylamide gel stained for protein as a guide, the ferritin-containing regions were removed as 2-mm gel slices from unstained gels. These slices were homogenized in a Thomas grinder with 0.5 cc PBS and used for immunization.

Immunization of rabbits.—Rabbits were immunized in the footpads and subcutaneously twice a week for 3 weeks. The initial immunization contained 6 homogenized gel slices (<300 μg protein) and 0.5 ml Freund's complete adjuvant. Subsequent immunization contained only the homogenized gel. Rabbits were bled from the ear 10 days after completing the schedule. Antibody-containing serum was also collected after booster immunizations at various intervals.

Immunodiffusion (ID), immunoelectrophoresis (IEP), and counter current electrophoresis (CEP).—Barbital buffer pH 8.2, ionic strength 0.075, was used to make 0.6% agarose plates. These were used for most immunologic procedures. For ID, 0.5 cm wells placed 0.5 cm apart were used. Inoculated plates were incubated at 4° C until precipitates appeared, usually in 24 hours. CEP was performed on 2×3 inch plates with parallel rows of 3 mm wells placed 3 mm apart, by use of 8 V/cm for 2 hours. Relative electrophoretic mobility values (m_r) were determined in 1% agarose, pH 8.4, ionic strength 0.05, with human albumin and transferrin used as standards and dextran for zero reference (*15*).

Staining.—Gels were stained for protein with Coomassie blue in 10% trichloroacetic acid (TCA) and for ferric iron with 2% potassium ferrocyanide in 0.1N HCl. Glycoproteins in acrylamide and agarose gels were stained by the periodic acid-Schiff (PAS) method (*16*). Haptoglobin in gels was demonstrated after saturation with hemoglobin by the *o*-dianisidine method of Ferris et al. (*17*). Ceruloplasmin was stained with *p*-phenylenediamine as a substrate (*18*).

Gel filtration and chromatography.—Gel chromatography was performed on Sephadex G-200. Column dimensions were 2.5×100 cm and the eluting buffer was PBS. Void volume was determined by blue dextran 2×10^6 molecular weight.

Immunofluorescence.—Ferritin labeling in tissue sections was done by the indirect immunofluorescence method; tissue was fixed in cold 100% ethanol, cleared in xylene, and embedded in paraffin as described by Sainte-Marie (*19*). Serial 5μ sections were then used for fluorescence studies. Sections were incubated with rabbit antiferritin sera that had been made specific by serial absorption with serum γ-globulin, red blood cells, leukemic myeloid cells, and lymphocytes cultured from Hodgkin's tumor nodules. Fluorescein-labeled goat antirabbit γ-globulin absorbed with human spleen powder was used as the indirect marker.

RESULTS

The pH 3.2 extract prepared from homogenized splenic tumor nodules separated into 3 protein bands on disc acrylamide electrophoresis. Antisera made against the protein in the upper portions of the small pore gel contained precipitating antibodies to 2 homogenate antigens. Although these 2 antigens migrated in the same region on acrylamide, they could be separated by IEP on agarose. One component migrated toward the cathode and showed complete IEP identity with normal human IgG. The other antigen migrated toward the anode (α-2 region) and stained strongly positive for ferric iron. This latter precipitation band did not identify with transferrin or any other serum protein (fig. 1), but showed complete CEP and ID identity with crystalline human ferritin (fig. 4).

The antiserum was rendered specific for ferritin by absorption with either normal serum or ammonium sulfate-precipitated γ-globulin. Antisera dilutions of 1:4 were used in CEP for detecting serum ferritin. The minimal concentration of ferritin detectable by this method was 0.4 μg ferritin protein/ml.

Tumor homogenates were filtered on Sephadex G-200 with the use of the specific antiserum to identify the fractions containing ferritin. The emergence of ferritin in the void volume behind the blue dextran marker indicated a molecular weight of between 2×10^5 and 1.2×10^6, consistent with the known apoferritin/ferritin molecular weight of 4.6×10^5 to 9×10^5 (*20, 21*). After chromatography on Sephedex G-200, the fraction having the highest titer of ferritin (precipitating activity at 1/640 dilution) was electrophoresed on acrylamide. After staining for either protein or iron, a weak band and a single strong band were present in the upper gel (fig. 2). This double band was consistent with reported electrophoretic properties of ferritin monomers (lower band) and dimers (upper band) (*22*). Additional stains for carbohydrate, haptoglobin, and ceruloplasmin were negative. Homogenized tissue supernatants from a variety of normal organs were tested by ID for the presence of ferritin. Normal spleen, liver, thymus, and placenta homogenates contained the greatest concentration of ferritin. Prostate, muscle, and heart contained smaller amounts.

Tissue Labeling

By immunofluorescence microscopy, antiserum specific for ferritin was found chiefly in the stroma of tumors and normal tissues and in the cytoplasm of monocytic and polymorphonuclear leukocytes. Staining of polymorphonuclear neutrophils was removed by absorption of antisera with white blood cells from chronic myelogenous leukemia, but this absorption did not reduce precipitating activity against ferritin (fig. 3). In nodular-sclerosing (NS) Hodgkin's disease fluorescence was found in the cytoplasm of phagocytic iron-laden histiocytes and in many of the spindle-shaped, fibroblastic-like cells found in the peripheral sclerosing areas of tumor (figs. 5, 6). In the mixed-cellularity (MC) form of the disease, the antigen was found in the same cell types but more diffusely distributed (fig. 7). Fluorescence staining of lymphocytes, Reed-Sternberg cells, atypical mononuclear cells, and lacunar cells was very faint and could not be distinguished from that present in control sections. In normal spleen sections, only cells lining the sinusoids stained, but we were unable to tell whether these were sinus histiocytes or endothelium. Cytoplasmic fluorescence was seen in placenta endothelium within chorionic villi and in thymic mesenchymal cells which encompassed the lymphoid follicles. In all cases fluorescence could be eliminated by absorption with ferritin.

Serum Presence of Ferritin in Hodgkin's and Other Diseases

Pretreatment sera from 108 patients with Hodgkin's disease were examined for the presence of ferritin: CEP was used for detection and identity (fig. 4). Forty-four percent of all patients with Hodgkin's disease had detectable serum ferritin. Table 1 shows the relation between stage and type of disease.

Table 1.—*Relationship of ferritinemia to type and stage of Hodgkin's disease**

Stage	LP	NS	MC	LD	Unclassified
I	0/0	4/7(40%)	0/2	0/0	1/0
II	1/1(50%)	13/27(32%)	1/4(20%)	0/0	
III	0/0	15/10(60%)	0/7	1/0	
IV	0/0	11/2(85%)	0/1	0/0	

* *Note*: Positive/negative (% positive); LP, lymphocyte predominance; and LD, lymphocyte depletion.

Table 2.—*Presence of ferritin in the blood of patients with malignant and nonmalignant conditions*

Disease	Number	Positive	Percent positive
Hodgkin's disease	108	47	44
LP	2	1	50
NS	89	43	48
MC	15	1	7
LD	1	1	100
Unclassified	1	1	100
Non-Hodgkin's lymphomas	32	8	25
Lymphocytic lymphoma			
Well differentiated	3	0	0
Poorly differentiated	10	5	50
Histiocytic lymphoma	10	1	10
Mixed histiocytic-lymphocytic	7	1	14
Others	2	1	50
Nonlymphoma malignancies (metastatic)	45	8	18
Nonmalignant disease	60	17	28
Viral pneumonia	16	5	31
Infectious mononucleosis	10	0	0
Herpes zoster	3	3	100
Hepatitis	15	9	60
Syphilis	5	0	0
Myocardial infarction	5	0	0
Dermatomyositis	1	0	0
Miscellaneous	5	0	0
Normal sera, free of disease	100	0	0
Fetal sera	10	0	0

In 28% of 32 pretreatment patients with non-Hodgkin's lymphoma, the serum contained CEP detectable ferritin. Table 2 shows the distribution of lymphoma type.

Forty-five patients with other forms of malignancies were studied. All of the tumors were metastatic. Eight sera contained ferritin. These were from patients with: acute leukemia (3), multiple myeloma (2), carcinoma of the breast (1), carcinoma of the prostate (1), and nasopharyngeal carcinoma (1). In patients hospitalized with a variety of nonmalignant conditions, there was a high incidence of ferritinemia in those with hepatitis, herpes zoster, and viral pneumonia (table 2). Normal sera were obtained from healthy volunteers, blood donors, and patients hospitalized for benign surgical conditions (hernias, varicose veins, etc.). Fetal sera were obtained from cord blood. All normal and fetal sera were negative.

DISCUSSION

Although nonspecific with respect to Hodgkin's disease, serum ferritin levels detectable by CEP are abnormal, and as such, could be useful in assessment of Hodgkin's disease activity. As our initial intent was to isolate and characterize antigens present in homogenates of Hodgkin's disease, we did not identify the tumor-associated protein as ferritin until late in the investigation. Antisera to ferritin were produced in rabbits by use of μg quantities of ferritin contained in acrylamide gel slices for the immunizing agent. These antisera, although specific, were less sensitive in detecting ferritin than those used in techniques reported by others (0.4 μg protein/ml. vs. 0.09–0.24 μg/ml) (*11, 23*). For this reason the results of our serologic survey probably represent a conservative estimate of hyperferritinemia.

In this study ferritin was detected in the serum of 44% of 108 patients with Hodgkin's disease. In patients with Stages III and IV NS-type disease, the incidence was 68%. Of 15 patients with MC Hodgkin's disease, however, only 1 had ferritinemia. He had Stage II disease with splenic involvement, and all of his disease was below the diaphragm. None of the 8 patients with Stages III and IV MC disease had hyperferritinemia. This latter finding is as yet unexplained. Tissue homogenates from both MC and NS Hodgkin's disease contained ferritin, and immunofluorescence studies demonstrated ferritin in both subtypes, although the quantity appeared to be higher in NS.

Difficulty was encountered in attempts to localize ferritin in tissue sections. Cryostat sections for indirect immunofluorescence studies were not technically adequate for morphologic study. The histology was improved by the paraffin-embedding technique of Sainte-Marie. By this method, the antigenicity of ferritin/apoferritin and its apparent product hemosiderin were preserved, but ferritin-specific fluorescence was less intense than that seen in frozen sections. Lymphocytes cultured from Hodgkin's disease tumors were also examined for ferritin by the indirect immunofluorescence method. Cytoplasmic staining in these cells was no greater than in controls using cells, incubated with serum of a nonimmunized rabbit. This finding most likely relates to nonspecific staining frequently encountered in immunofluorescence work. Homogenates of these lymphocytes had no CEP-detectable ferritin even after stimulation with ferric ammonium citrate. This latter technique reportedly increases ferritin production by other tumor-derived cell lines (*24*).

In other reports describing circulating ferritin in Hodgkin's disease, the investigators stress its relationship to extensive disease (*10, 11*). Although our study is consistent with this idea, it does not account for the 32% incidence of detectable serum ferritin in untreated patients with surgically determined Stages I and II disease. In these patients, localized tissue necrosis probably caused release of intracellular ferritin into the serum; yet in patients with other inflammatory or necrotizing diseases, such as myocardial infarction, decubitous ulcer, appendicitis, or syphilis, ferritinemia was not detected. It cannot be concluded then that necrosis and extent of disease are the only mechanisms responsible for hyperferritinemia in Hodgkin's disease.

In a subsequent preliminary study, the presence of ferritin in the serum of a small group of treated patients completely correlated with clinical evidence of continued disease. Therefore we feel, as does Aungst, that the presence of small amounts of ferritin in the circulation might constitute a sensitive index of activity in Hodgkin's disease. In this study, only serum ferritin levels >0.4 μg/ml were detectable; yet the range of normal values as determined by radioimmunoassay is 0.01–0.10 μg protein (*8*). By use of radioimmunoassay, additional patients with ferritinemia will undoubtedly be recognized. We are currently using a "two site" radioimmunoassay for determination of ferritin levels prior to, during, and after therapy (*25*).

These studies will be reported upon completion of the protocol.

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FIGURE 1.—Presence of ferritin in serum demonstrated by IEP in 0.6% agarose. *Upper well* contains normal serum; *lower,* serum from patient with Hodgkin's disease. *Upper* and *lower troughs* contain antihuman sera. *Middle trough* contains antisera specific for ferritin. Precipitin-band forms in α-2 region.

FIGURE 2.—Position of tumor proteins electrophoresed on 7.5% acrylamide gels. From *left to right*: 3.2 pH extract of Hodgkin's tumor nodules, purified tumor ferritin, and ferritin stained for ferric iron. Double band of ferritin corresponds to monomers (*lower band*) and dimers (*upper band*).

FIGURE 3.—CEP of a standardized ferritin solution in normal serum (*upper wells*) against ferritin antisera serially absorbed with the following: 1) unabsorbed, 2) human γ-globulin, 3) human red blood cells, 4) cultured lymphocytes from Hodgkin's tumors; 5) leukemic white blood cells, and 6) purified ferritin.

FIGURE 4.—CEP of patient serum (*upper numbered wells*) against specific rabbit antiferritin (*bottom wells*). *Wells 1–4* contain serum from patient with NS Hodgkin's disease; *well 5,* MC; *well 6,* normal serum; ferritin standard, in wells lateral to numbered wells is used for identity reactions.

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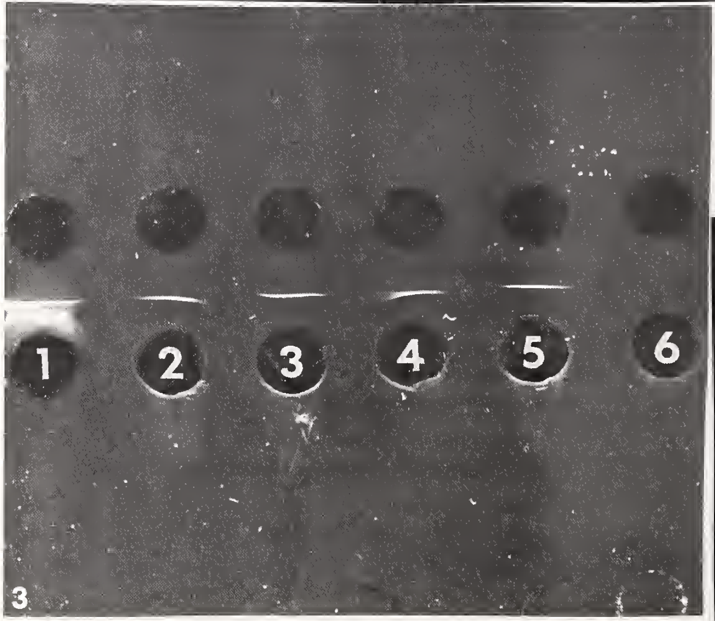
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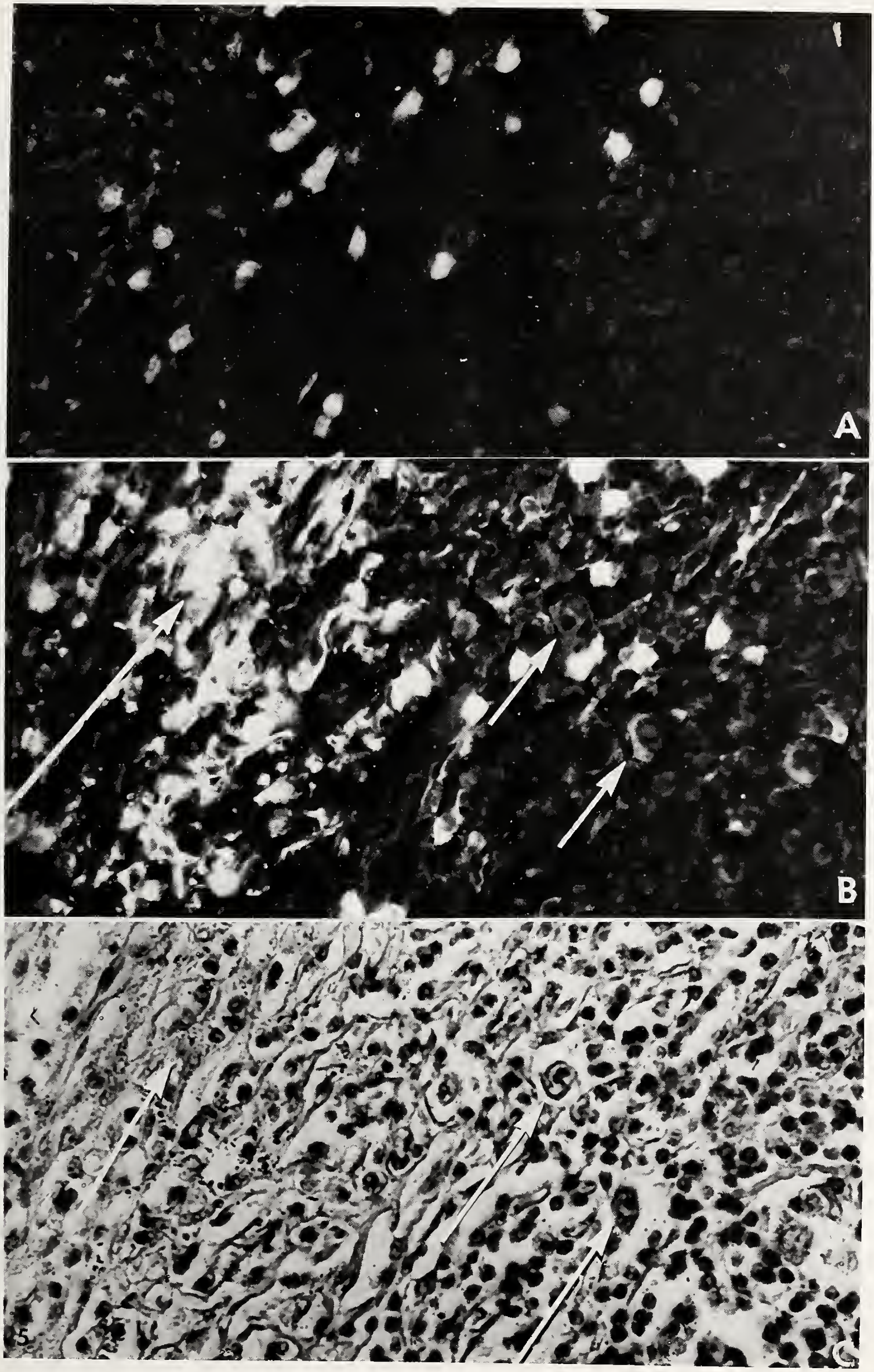

Figure 5.—Immunofluorescence photomicrographs (*A,B*) of Hodgkin's disease NS splenic tumor nodule. *A*) No fluorescence when stained with antisera absorbed with ferritin. Illuminated cells are eosinophils transmiting blue light. *B*) Fluorescence of ferritin antigens by specific antisera. *Arrows at left* denote atypical mononuclear cells. *C*) *B* restrained with hematoxylin and eosin. *Arrow at right* indicates several cells containing hemosiderin. Indirect immunofluorescence. × 450

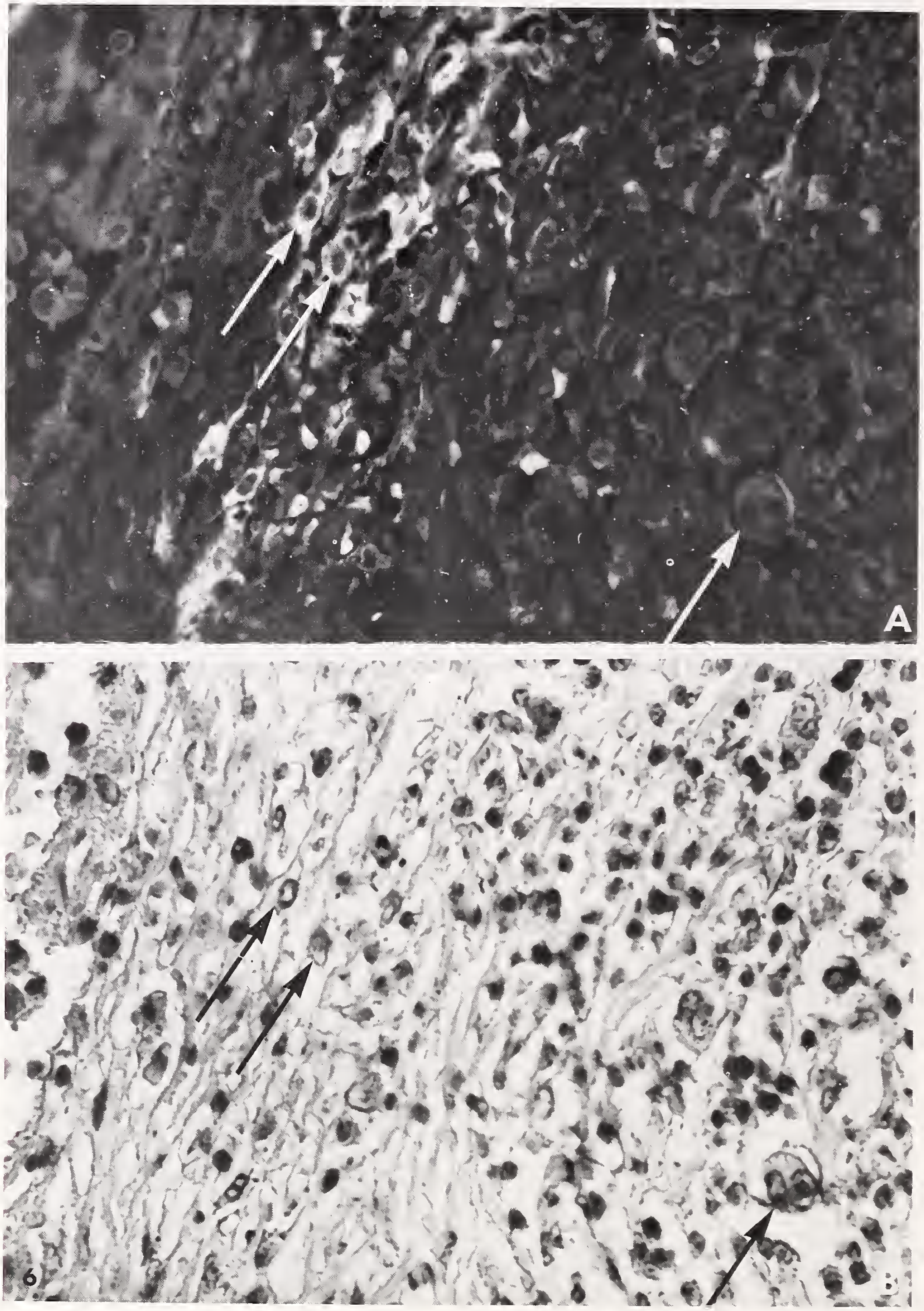

FIGURE 6.—Three-micron section of Hodgkin's disease splenic tumor nodule: *A*) stained for ferritin antigens and *B*) poststained with hematoxylin and eosin. *Arrows to left* mark atypical mononuclears and Reed-Sternberg cells. *Arrow to right* in the sclerotic tumor margins denotes cells staining for ferritin. Indirect immunofluorescence, × 450

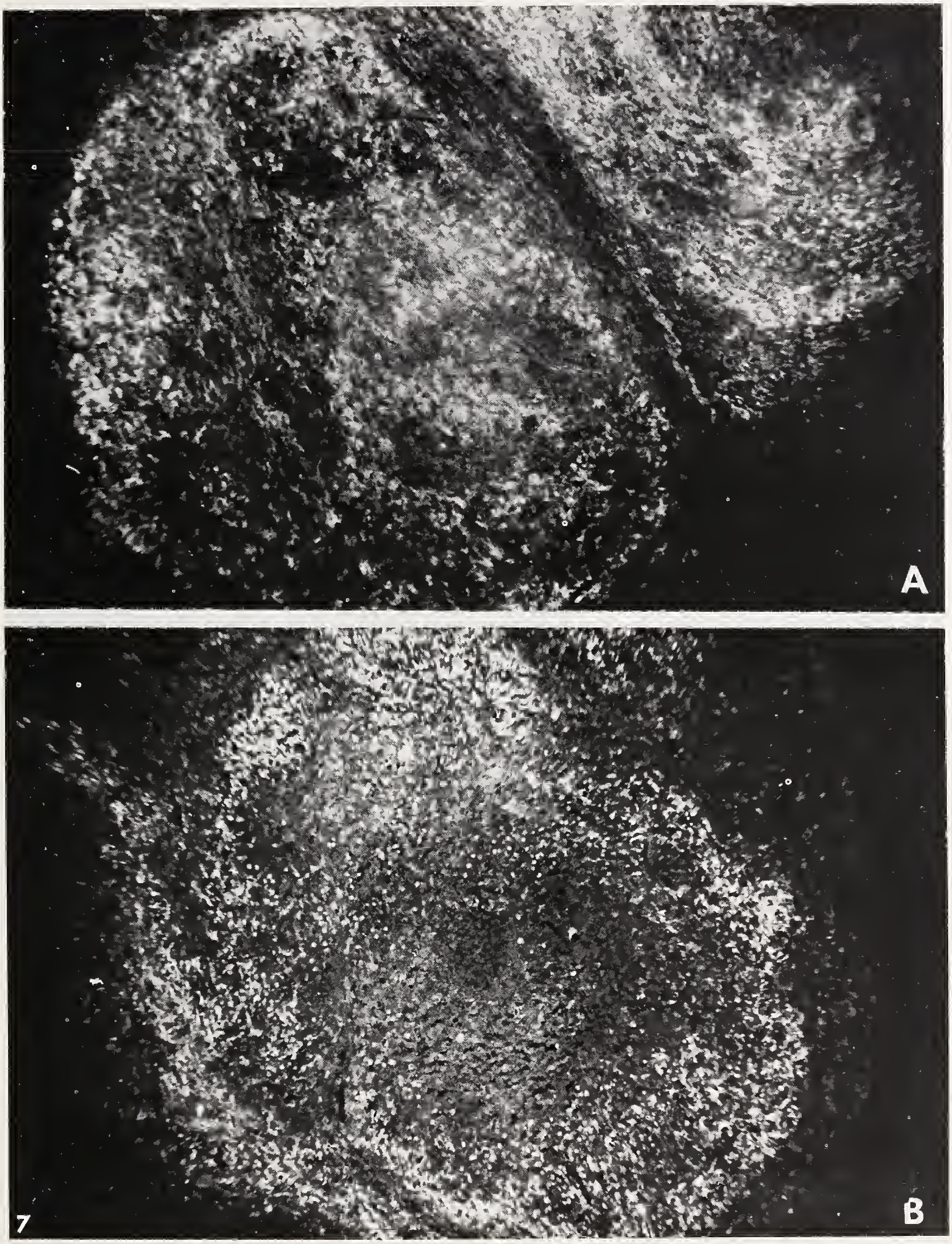

FIGURE 7.—Fluorescence photomicrograph of Hodgkin's disease splenic tumor nodules from patients with *A*) MC and *B*) NS. Illumination of nodules is due to binding of specific antisera to ferritin antigens and transmission of blue light ("autofluorescence") by eosinophils. Indirect immunofluorescence. × 100

Invited Discussion: Approaches to the Serologic Characterization of Hodgkin's Disease[1]

E. Sega, F. Isabella, M. Minnetti, T. Petrucci, ***and*** **G. Citro,**[2] ***Laboratorio di Immunologia, Istituto Regina Elena per la Cura e lo Studio dei Tumori, Viale Regina Elena, Rome, Italy***

POLYACRYLAMIDE GEL electrophoresis (PAGE) of serum proteins permits the separation of approximately 20–30 components (*1, 2*) in contrast to the 5 or 6, which can be detected by cellulose acetate electrophoresis, usually employed for clinical purposes.

Isoelectric focusing with polyampholytes in stable pH gradient is a fractionation method with very high resolving powers, great operational simplicity, and maximal reproducibility; thus it represents a further improvement in protein analysis (*3, 4*). The procedure has been modified for use with polyacrylamide gel media (*5–10*). These techniques make the detection of differences between normal and pathologic serum patterns more feasible.

This study uses the new techniques to compare normal and Hodgkin's sera with the aims of detecting their differences and separating any "abnormal" protein fraction.

With these "abnormal" proteins, a "specific" antiserum could be prepared and normal and pathologic sera could be tested by immunodiffusion or disc immunoelectrophoresis against these "specific" antisera.

MATERIALS AND METHODS

Individual sera and pools of 20–30 sera from normal subjects and from patients with Hodgkin's disease were used. PAGE was performed according to Ornstein's method (*11*) as modified by Sega et al. (*12*). For electrofocusing, the polyacrylamide medium was combined with a 2% ampholyte solution (Ampholine LKB 40%). The upper bath was filled with 5% phosphoric acid solution and the lower bath with 5% ethylenediamine solution. A starting current of 400 V was applied for 1 hour for ampholyte migration; the current was then lowered to approximately 0.5 ma per gel column. After the run, the gel cylinders were fixed in 10% trichloroacetic acid solution and recorded in a Gelmann spectrophotometer at a wave length of 280 mμ. Selected protein fractions were sliced off, homogenized in phosphate-buffered saline (PBS), and injected with Freund's adjuvant into rabbits. Normal sera and sera from patients with Hodgkin's disease were tested by disc immunoelectrophoresis and by immunodiffusion in agar against the antisera obtained from the immunized rabbits. Antisera from rabbits immunized against selected protein fractions, detected in Hodgkin's sera, were adsorbed with normal sera and tested as described above against normal and Hodgkin's sera.

RESULTS

PAGE patterns of normal and Hodgkin's sera are shown in figure 1. Hodgkin's serum bands in all migration zones (postalbumin, posttransferrin, slow-globulin, and haptoglobin-macroglobulin) seem more numerous. This deviation from normal is particularly evident in the haptoglobin-macroglobulin zone. Elution of this zone in PBS was followed by a PAGE run of 6 normal and 6 Hodgkin's sera and gave the spectrophotometric patterns shown in text-figure 1. The variability of the patterns in both normal and Hodgkin's sera probably indicates

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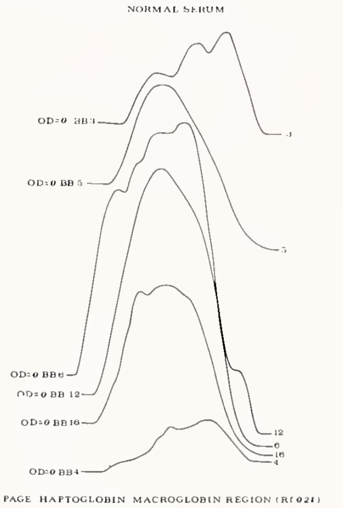
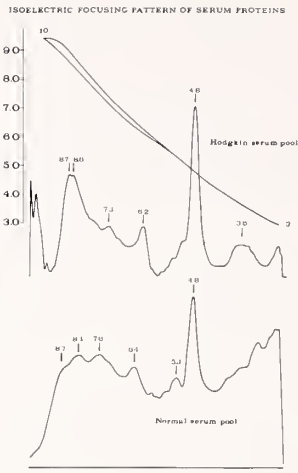
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] We gratefully acknowledge Mr. Luigi Dalloco for skillful technical assistance.

genetically controlled proteins, as is due to haptoglobin types (*1*). Several pools of 20–30 normal and Hodgkin's sera were prepared to lower the concentrations of the single, genetically controlled bands and increase the concentrations of any abnormal ones present in Hodgkin's sera. The pherograms of Hodgkin's serum pools showed 2 sharp bands in the haptoglobin-macroglobulin region not seen in the same region of normal sera pools (fig. 2). Electrofocusing after fractionation with ammonium sulfate also showed differences between normal and Hodgkin's serum pools (text-fig. 2).



Text-figure 1.—Spectrophotometric recording of 6 normal sera (only haptoglobin-macroglobulin region). Individual variability is also shown in normal sera due to the genetically controlled protein fractions (in particular, haptoglobin).



Text-figure 2.—Electrofocusing of normal and Hodgkin's serum pools. Hodgkin's serum pool shows many more proteins with different isoelectric points than found in normal serum pools.

The region of Hodgkin's serum pools containing the abnormal bands was isolated and eluted with PBS. The eluate was used to immunize New Zealand White rabbits. A similar procedure was carried out for comparison on the corresponding region from normal serum pools. Disc immunoelectrophoresis of individual normal and Hodgkin's sera was done with the 2 antisera.

The antiserum from animals immunized with the eluate from Hodgkin's serum pools was used against a Hodgkin's serum. Five precipitin arcs were obtained. The first was in the postalbumin region, the second and third in the transferrin region, the fourth in the slow-globulin region, and the fifth in the haptoglobin-macroglobulin region. The same antiserum used against a normal serum produced only 3 arcs: in the postalbumin, transferrin, and slow-globulin regions.

Twenty normal sera from blood banks and twenty samples from unselected Hodgkin's disease were analyzed with double immunodiffusion in agarose against rabbit anti-Hodgkin's pool serum adsorbed with normal serum.

No precipitin lines were observed with normal human sera. However, the rabbit antiserum reacted with Hodgkin's sera in 85% of the cases (fig. 3).

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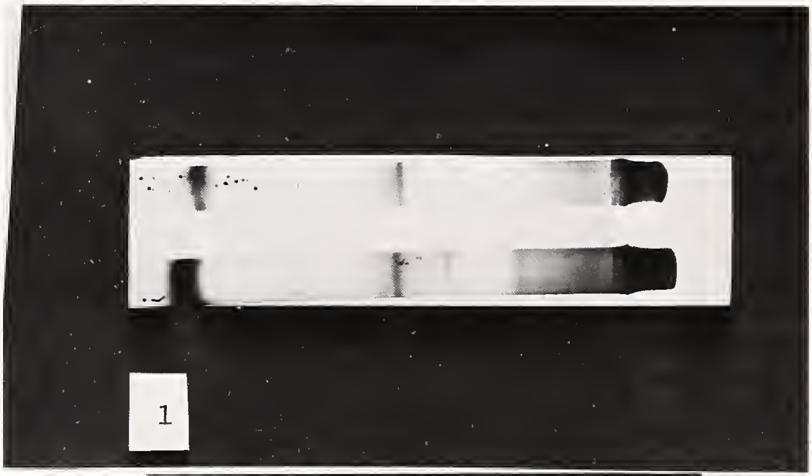

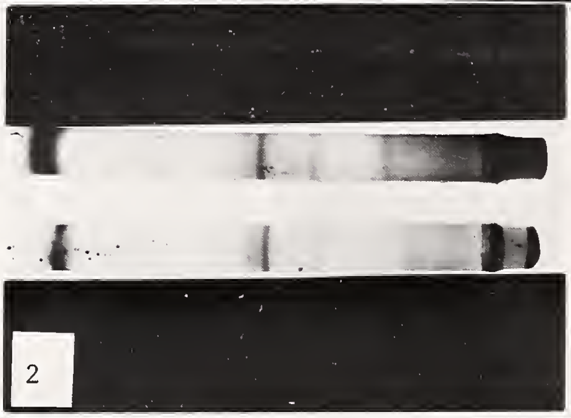

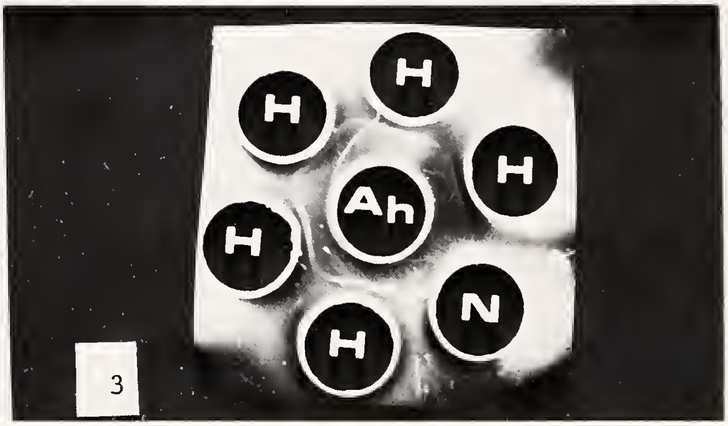

Figure 1.—PAGE pattern of normal (*lower*) and Hodgkin's sera (*upper*). Deviations from normal are particularly evident in haptoglobin-macroglobulin zone.

Figure 2.—PAGE pattern of normal serum pools and Hodgkin's serum pools, with significant differences. *Note* 2 sharp bands in haptoglobin-macroglobulin region.

Figure 3.—Agar immunodiffusion (Ouchterlony double immunodiffusion). *Central well*: Antiserum from rabbits immunized with the eluate from Hodgkin's serum pools and absorbed with normal serum pool and AB-donor blood cells. *Peripheral wells*: H = sera from Hodgkin's disease patients; and N = serum from normal patients.

Invited Discussion: Tumor-Associated Antigen in Hodgkin's Disease [1]

Michael J. Peckham, ***M.A., M.D., F.F.R., Institute of Cancer Research, The Royal Marsden Hospital, Sutton, Surrey, England***

EVIDENCE for a tumor-associated antigen in Hodgkin's disease reported in 1971 by Order, Porter, and Hellman (*1*) was of considerable interest to us, both for the etiologic implications and, on a more practical level, the possibility which it offered to develop a method of monitoring remission patients for the early detection of relapse.

A similar study was initiated in our laboratories by Dr. Pamela Denton. Seven antisera have been raised in rabbits against Hodgkin's tissue by the intramuscular route for immunization and Freund's adjuvant for 3 of the 4 injections of tumor material. In the first 2 experiments, water extracts of pooled nodal and splenic tissue, one nodular sclerosis and the other mixed-cellularity Hodgkin's disease, were used. Five antisera were raised against whole cell suspensions. The antisera were absorbed against splenic tissue, AB red cells, and serum from individuals without malignant disease.

Preliminary indirect immunofluorescence on cryostat sections has encountered problems from autofluorescence, but despite this, heavy pericellular staining was observed in all Hodgkin's tissue examined so far. Immunofluorescence on free cell suspensions has not yet been attempted, though this may enable staining to be related to cell morphology, especially if cell separation techniques can be developed.

Immunodiffusion studies with anti-mixed-cellularity and anti-nodular-sclerosis antiserum (prepared against water-extracted Hodgkin's tissue) demonstrated a single line against the antigenic material and against water extracts of Hodgkin's tissue from 8 patients with a variety of histologic grades. Immunodiffusion against normal splenic tissue showed no line. The antisera were run against sera from 30 patients with a variety of conditions. Two of ten Hodgkin's disease patients were positive, but some of this group were on chemotherapy and it is clear that this aspect needs to be extended and clarified. Seven normal sera were negative. Of 3 patients with infectious mononucleosis, 2 had positive sera and both showed a single band. Of 9 patients with various forms of malignant disease, 2 were positive, one with acute myeloid leukemia and the other with a carcinoma of the rectum. The precipitation lines, not fully characterized, resembled those identified in Hodgkin's disease.

More recently, we have used immunoelectrophoresis as described by Order, Chism, and Hellman (*2*). This work is in the early stages but our preliminary observations agree with the findings at the Harvard Medical School.

The results obtained so far on tumor-associated antigen in Hodgkin's disease have encouraged us to continue and develop this work further to determine whether the antigen is related specifically to Hodgkin's disease or whether it can be identified in benign, actively proliferating lymphoid tissue such as reactive hyperplasia. Histologically normal lymphoid tissue removed from Hodgkin's disease patients at laparotomy will be examined in relation to the findings on tumor tissue from the same patient. Finally, it will be of interest to ascertain whether there is any cross-reactivity with tumor-bearing lymphoid tissue from patients with the non-Hodgkin's lymphomas.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

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Invited Discussion: Tumor-Associated Antigens and Hodgkin's Disease[1]

Karl Erik Hellström, *Department of Pathology, University of Washington School of Medicine, Seattle, Washington 98105*

I FIRST want to give a background in tumor immunology; since it has to be short, I am omitting the references except for a recent review covering most of the aspects touched upon (*1*).

We know from work done since the mid-1950's that most (and maybe all) animal tumors have antigens that either do not occur in normal cells at all, or occur in much smaller quantities. Animals can be immunized so that they become more resistant to the transplantation of cells that contain given tumor-specific antigens. We also know that there is some kind of immunologic surveillance against tumors in vivo because, if one suppresses immunologic reactivity by performing neonatal thymectomy or by treating animals with antilymphocytic serum or other immunosuppressive agents, more primary tumors generally develop. Most of us also hold a similar view with regard to human tumors: Cell-mediated and humoral immunity to human neoplasms has been detected, and patients who are immunodeficient from birth or after immunosuppression (as, e.g., kidney graft recipients) develop more tumors—not only leukemias and lymphomas, but also other types of tumors. One of the more interesting observations made on the human neoplasms is that histologically similar tumors cross-react antigenically, while tumors of different types do not cross-react. The presence of common antigens among a group of similar neoplasms does not exclude that the same tumors can also have individually unique antigens; evidence for malignant melanoma shows that this is, indeed, the case.

One may ask whether antigenic differences between a tumor and its host can serve clinically useful purposes. First, we turn to immunotherapy in animal models. In tumors with strong, specific antigens, transplanation of tumor cells can be prevented by proper immunization of the animal. One can sometimes achieve a relatively modest immunotherapeutic success with already established tumors by the transfer of specifically immune lymphocytes or RNA from properly immunized donors, or by inoculation of specific antigens intradermally, or by nonspecifically stimulating the reticuloendothelial system with BCG (bacille Calmette-Guérin). Recent studies indicate that cytotoxic sera having so-called "unblocking" properties can neutralize the blocking effect detected in most sera from tumor-bearing animals (as an abrogation of specific cell-mediated tumor immunity), and can elicit an immunotherapeutic effect. (The in vivo effect of such sera may be due to their unblocking activity, or may have some other explanation.)

As for prevention of tumors, it is much too early to speculate whether the common antigens in human tumors of the same type can make possible vaccination against some groups of human neoplasms. It has been shown, however, that some animal tumors with common antigens can be prevented by immunologic means.

Immunodiagnosis, finally, has already been attempted in man, with some success. The work by Gold and associates (*2*) has given clues that may help to diagnose colonic carcinomas, since the carcinoembryonic antigen (CEA) characterizing such tumors was found in the serum of patients with growing colonic carcinoma. The early work on the diagnostic aspects looked promising, with very few false positives and false negatives (*2*). Lately there have been challenges to the specificity of the CEA of colon cancer, however (*3*). In particular, there have been quite a few false positives in cases of alcoholic liver cirrhosis and in certain other tumors like carcinomas of the lung. Thus despite much work, we do not yet know to what extent immunologic assays will be helpful for routine diagnosis of colonic cancer. An important beginning has been made, however, and immunologic techniques will probably be helpful in monitoring individual tumor patients for the effects of tumor therapy, even if the possibility for mass screening of large populations is more remote.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

We now turn to the subject of this meeting, Hodgkin's disease, and to what extent evidence exists for immune reactions against cells characteristic of that disease. The study by Order et al. (*4*) shows that some cells in Hodgkin's disease have new antigens, though the data do not indicate that the antigens are tumor specific. At most, the antigens can be called tumor associated. The value of these antigens for diagnosis will be determined by the extent to which one can develop simple and reproducible assays for them (or for antibodies to them, if such exist), and by the extent to which one can then detect patients with Hodgkin's disease, without getting too many false negatives or false positives. Indeed, I was pleased with the careful way the work of Order et al. was done, without claims that the antigens were more specific than the data justified. It is important that clinical studies to find the diagnostic potential of the immunological assays are done in a double-blind fashion, so that one does not get carried away with unwarranted enthusiasm.

Whether the antigens described by Order et al. are of value for tumor prevention and therapy is difficult to say. Personally, I would be surprised if these antigens were good targets for tumor prevention or therapy, since they occurred also in several cases not involving Hodgkin's disease (e.g., in a patient with hiatus hernia). One should nevertheless keep an open mind for the possible therapeutic implications.

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Part 4. Synthesis and Epidemiologic Implications

Invited Discussion: Synthesis and Epidemiologic Implications [1]

Marvin A. Schneiderman, *Associate Scientific Director for Demography,*
National Cancer Institute,[2] *Bethesda, Maryland 20014*

TO START this discussion, let me propose an hypothesis about this disease and an hypothesis of how to relate it to some of the things we have heard at this meeting. We are dealing with a disease to which only a very small proportion of the population is susceptible, probably in some genetic sense. The HL-A antigen work that Bodmer (*1*) and others have presented indicates this pattern of susceptibility. As Bodmer points out, these markers are probably not the best things to measure but today they are the best we have to measure. This susceptibility is modulated, modified, or moderated by something that exists in our society, in our way of life, and within the individual in addition to his susceptibility. What are the implications of this notion? Insofar as the society may be modifying something in the susceptible individual, we must look to such things as viruses and other possible carcinogens in the world about us. We must also look to some factor related to a possible infectious agent. There is a vast amount of data which seem to point in this direction.

I looked at the male-female ratio for Hodgkin's disease in the United States and at the male-female ratio for deaths from infectious diseases. The two ratios were the same for the whole U.S. population. Something is related there, but what it is obviously I do not know.

What are the implications of this hypothesis from the social point of view? If it is something in our way of life that produces high levels of this disease in childhood, perhaps because of inadequate nutrition, or perhaps because some defects occur in the general immunologic development of children, we have to recognize that persons who have those defects are not only going to develop Hodgkin's disease; they are going to develop other diseases and they are going to die from these other diseases. Thus there are going to be fewer of these people around in the adult age group, and we are going to have less of the disease in young adults in a population in which many people develop or can develop that disease in childhood. I am a little more in agreement with D. W. Smithers and with G. T. O'Conor than I am with my colleague and friend, Brian McMahon. Hodgkin's disease could be a single disease with different cohort manifestations in the population.

What kinds of epidemiologic studies are needed? We have to do more migrant studies. What happens to the people who have moved to different countries where the social levels are different and the ways of living are different? Does the incidence of the disease then move toward the situation in the country to which the people have migrated? We have to look at trends in time. If this is a disease related to some kind of social set—if it is a disease of high social levels—then we ought to be seeing an increase in this disease as time goes on in those countries in which the level of living is increasing. We are seeing such a phenomenon. Dr. Stalsberg (*2*) showed increases in Hodgkin's disease in almost all the European countries. This is consistent. If we are doing things in our society which are protecting young children from the disease in their youth, we may be protecting them until they reach the age of fertility and, therefore, we are going to continue these *HL-A* susceptibility genes in the population and that, too, should give us an increase in Hodgkin's disease. Do the various histologic types of the disease—nodular sclerosis, lymphocyte depletion, lymphocyte predominance, and mixed cellularity—have anything to do with etiology? If we think they do, we must ask one more question: "What is different about Connecticut and California in the ways of living?" If not due to ways of living, how do we account for the different distributions of histologic type in the two areas? The California data appeared to be 75% or 80% nodular sclerosis, whereas in the Connecticut data that proportion was reportedly very much lower. I would like to see a population-based study developed through the California Tumor Registry, with a biopsy slide review, just as was done in Connecticut. Perhaps we have an unusual sample here at Stanford University Medical Center in the kinds of patients who have come to Henry Kaplan and his staff. Maybe there is not that large a difference between Connecticut and California as a whole.

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[1] Presented at the International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Finally, concerning the 2 or 3 peaks in the age-specific incidence curves, when we look at all cancer in the United States in whites and blacks (in which there are certainly some genetic differences with a very obvious genetic marker), we think there are also some differences in the way of life. You will find, in looking at all cancers in U.S. whites, that cancer in general is a disease which starts low and comes up and continues to climb in incidence, with the very oldest age groups having the highest incidence. In blacks, cancer is not such a disease. It is a disease in which the levels are higher in the younger ages and then there is a falling off or maybe a flattening out at the higher ages. I think that this has something to do with other causes of death. This is why I first introduced the notion of the susceptible individuals in Hodgkin's disease. If there is a high level in early life and this reflects susceptibility to a whole flood of things, not just this disease, many more people are going to be removed from the population early who would normally have developed the disease at later ages.

What I heard today encouraged me enormously to feel that there are some patterns to this disease. The age distributions in different countries are meaningful and the fact that we seem to find age patterns like the age pattern from deaths due to infection is meaningful in helping work out the etiology of this disease.

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Invited Discussion: Synthesis and Epidemiologic Implications [1]

Robert W. Miller, *Epidemiology Branch, National Cancer Institute,*[2] *Bethesda, Maryland 20014*

WE have heard at this Symposium descriptions of variations in the frequency of Hodgkin's disease according to cell type and of tremendous variations in different geographic areas according to age and sex. What we heard exemplifies the power of geographic pathology. If one considers Hodgkin's disease as an entity without splitting it into its component parts, progress in determining its causes is likely to be slowed. Differences in frequencies according to sex among people living in the same country or the same city may well represent differences in host susceptibility, with males more susceptible than females. It is strange that the magnitude of the differences varies according to age. It is now up to laboratory research workers to suggest hypotheses that might account for these differences and then to make studies to document their hypotheses if possible.

The descriptive studies presented exemplify only one category of epidemiology. Epidemiology can also generate or test hypotheses.

This brings us to the second part of the program. Is there a viral etiology in Hodgkin's disease? We know much more in this respect about leukemia than we do about Hodgkin's disease. Many hypotheses about the virus-like transmission of leukemia have been tested through epidemiologic research. Is it transmitted in the breast milk, for example? The answer appears to be no. Is it transmitted from the pregnant mother with leukemia to the child who was in utero at the time? The answer is no. Is it transmitted from one spouse to the other? Again the answer is no. We know far less about Hodgkin's disease.

There are two hypotheses about the viral etiology of Hodgkin's disease that have recently attracted considerable attention. One is based on Davies' studies which suggest that case-contact-case transmission of the neoplasm may lead to epidemics, such as he has reported in Albany, New York (*1*). One might ask if it is reasonable to expect that clusters of this sort would occur with an intermediary between the cases, when clusters of cases without intermediaries have not yet been demonstrated. It seems to me that the statistical techniques developed for case-clustering need to be applied to Hodgkin's disease to determine if excesses of clusters in time and space can be demonstrated.

Another question of particular interest to virologists concerns the relationship of infectious mononucleosis to leukemia and lymphomas. We did a study based on infectious mononucleosis in veterans in relation to the subsequent development of cancer of any sort (*2*). We made the study in two ways. One component was prospective; i.e., we studied 2,437 white male veterans who served during World War II in the Army and who developed infectious mononucleosis during their military service. We followed all of them and an equal number of matched controls forward in time to determine the causes of death among the 107 who died. The other part of the study was retrospective: We looked back through the military records of 2,659 veterans of World War II who died of cancer to determine if they had infectious mononucleosis diagnosed during military service.

Review of the cases revealed that generally the diagnosis of infectious mononucleosis was of good quality, though, of course, it was not perfect. In the prospective study, 8 of the men died of lymphoma or leukemia (4 with lymphosarcoma or reticulum cell sarcoma, 2 with Hodgkin's disease, and 2 with leukemia) as compared with 4 among the controls. The difference between these 2 numbers was not statistically significant. The difference was further diminished when the histories of these patients were studied. One man who was thought to have had infectious mononucleosis apparently had that diagnosis confused with the early stages of Hodgkin's disease. His heterophil antibody titer was never elevated, and he had no lymphocytosis. Another man in the prospective study who died of reticulum cell sarcoma also apparently had prodromata of the neoplasm confused with infectious mononucleosis. When these 2 cases were discounted, there were 6 veterans who died of leukemia or lymphoma following the diagnosis of infectious mononucleosis during military service as compared with 4 among the controls. The study was powerful enough to have detected at a 90% probability level a fourfold or greater increase in leukemia/lymphoma frequency. We did not find it.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

In the retrospective study, we found that, among 2,659 veterans who died of cancer, 6 had had infectious mononucleosis diagnosed during military service. One of these men had lymphosarcoma and another had plasma cell myeloma. Among the controls, 4 had infectious mononucleosis. Again, the difference between cases and controls was not statistically significant.

Finally, let me turn to another test of a hypothesis, this time in regard to immunology. All the circumstances that carry a high risk of leukemia are characterized by pre-existent chromosomal abnormality, though not of a single type: chromosomal fragility on cell culture in Bloom's syndrome and Fanconi's aplastic anemia, an extra chromosome in Down's syndrome, or chromosomes that have been broken by exposure to radiation, benzene, alkylating agents, etc.

By contrast, the pre-existing circumstances that occur in association with lymphoma are inborn immunologic deficiency states, acquired immunologic disorders, and immunosuppressed states produced by drugs. This arrangement must look familiar to Chairman Good (*3*), because he originated it, with an absolute minimum of cases. Probably no epidemiologist would have taken that risk. Good formulated his hypothesis on the basis of lymphoreticular neoplasia in 8 cases of ataxia-telangiectasia, 3 cases of Wiskott-Aldrich syndrome, and 3 cases of the Bruton type of congenital agammaglobulinemia. He hypothesized that these relationships signified lymphoma was related to cell-mediated immunologic deficiency, and he proved to be right. There are now dozens of cases of lymphoma with ataxia-telangiectasia or Wiskott-Aldrich syndrome, and the immunosuppressive drug-induced cases of lymphoma show that an exogenous agent can produce effects similar to those seen when immunologic deficiency is inborn.

Good's work also played a role in the definition of groups at high risk of leukemia. He was the first to show conclusively the relationship between this neoplasm and Down's syndrome. Again he did it through a study that today would probably not be approved for a grant because of imperfections in scientific design. Fortunately he did not need a grant. He did it by mail and it cost, I think, nine dollars.

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SUMMARY OF INFORMAL DISCUSSION ON SYNTHESIS AND EPIDEMIOLOGIC IMPLICATIONS

W. Henle questioned the reliability of the diagnosis of infectious mononucleosis (IM) in an Army and Veteran population in which the disease was not reportable. **R. Miller** replied that his data had come from an inventory of all disease that occurred during World War II as applied to the discharge diagnosis. It is true that there may have been infections that would not have been diagnosed correctly. Nonetheless, he was confident that this group was rich in people who had genuine IM while they were of military age. **F. K. Sanders** added a comment to reinforce what Doctor Henle had said. At Cornell University a recent study had documented the existence of a very high incidence of occult IM.

H. S. Kaplan stated that he had recorded approximately 50 cases of prior IM in his series of patients with Hodgkin's disease. All of these cases occurred in the adolescent and young-adult groups and represented approximately 20% of the Hodgkin's disease patient population in the 15- to 35-year-old age group. Contrary to one of Doctor Miller's statements, he had not seen a single case among >200 cases of Hodgkin's disease in that same age group who had developed IM *after* having Hodgkin's disease. To the best of his knowledge, there is only one such report in the literature. Therefore he challenged the statement that the 2 diseases occur in a random sequence. He asked what was the average interval in the Veterans' study between the time when these individuals reportedly had their IM and the subsequent tally of whether Hodgkin's disease had occurred or not. In the Stanford University series, the average interval was about 3–5 years. **Miller** replied that his study had run from 0 to 20 years after the diagnosis of **IM.**

D. Wright asked Doctor Kaplan to elaborate on the diagnostic criteria in his 50 cases of IM followed by Hodgkin's disease. He also asked Doctor Good to elaborate on the cases of lymphoma that have occurred in immunologic deficiency states, because it was his impression that they are predominantly lymphomas of the non-Hodgkin's variety. **Kaplan** replied that most of the diagnoses were based on a combination of clinical manifestations, peripheral blood cell smears, and heterophil antibody titers, but that some of the cases were very poorly documented. **R. A. Good** replied to Doctor Wright's question about the kinds of malignancies seen in the immunodeficiency-disease states. Ataxia-telangiectasia is a dual system immunodeficiency in which there is both a cell-mediated and humoral deficiency; about 70% of the patients lack IgA and about 80% lack IgE. To date, 47 of 430 of these patients have had malignancies. These have been mostly lymphosarcomas and "lymphoreticular sarcomas." There have been 3 that have been called Hodgkin's disease. What is needed so badly in this study is just what Doctors Rappaport and Dorfman have suggested—a panel of expert pathologists to review these slides. There is one family in which a child of 15 and a child of 17 or 18, both of whom had ataxia-telangiectasia, developed gastric carcinoma. That is more than one would expect by chance alone.

G. T. O'Connor commented that, in studies of prior IM in patients with Hodgkin's disease, what we really want to look at is seroconversion rather than clinically overt IM.

S. A. Rosenberg thought it surprising in a discussion of possible viral etiology of Hodgkin's disease, that no one had mentioned herpes zoster. We have always presumed that herpes zoster is a manifestation of the depressed immunity associated with the disease or its treatment, but at least some consideration should be given to a possible etiologic link. He asked how one could approach the problem of herpes zoster in patients with Hodgkin's disease. Could one compare it to other immunosuppressed patients?

SESSION 2

HISTOPATHOLOGY AND EXPERIMENTAL CYTOLOGY

Chairman: Ronald F. Dorfman

INTRODUCTION

There is an unfortunate impression that pathologists do nothing other than peer down microscopes and look at slides stained with hematoxylin and eosin and a few other stains just for interest's sake. This is a far cry from modern pathology. Our Department of Pathology at Stanford University Medical Center reflects the fact that our interests are very widespread. We have members of the department who are not pathologists, who are immunologists, molecular biologists, and so on. In planning this session, we felt that we did not want to repeat the purely histopathologic discussions that we have had on many other occasions. Instead, we would like to consider other, more fundamental aspects of pathology which bear on the biology of Hodgkin's disease.

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Cell Proliferation in Hodgkin's Disease[1]

Michael J. Peckham, *M.D., F.F.R., and* Edward H. Cooper, *M.D., D.Phil., Institute of Cancer Research, The Royal Marsden Hospital, Sutton, Surrey, England; and Department of Cancer Research, University of Leeds, Yorkshire, England*

SUMMARY—The proliferation characteristics in Hodgkin's tissue were studied in relation to cell morphology with the use of tritiated thymidine labeling and quantitative cytochemistry. Earlier studies with the use of in vitro labeling showed two populations of proliferating cells, one resembling normal lymphoid cells and the other mononuclear cells, characterized as Hodgkin's cells, which commonly showed evidence of aneuploidy. These observations were confirmed by direct labeling in vivo of involved nodes at laparotomy and Hodgkin's skin nodules. This technique revealed that a small proportion of Reed-Sternberg cells incorporated tritiated thymidine. The findings are discussed in relation to the overall growth pattern in Hodgkin's disease and the other lymphomas.—Natl Cancer Inst Monogr 36: 179–189, 1973.

THE CELL biology of Hodgkin's disease is complicated. There is at present only fragmentary evidence on the function, derivation, and interaction of the various lines of lymphoid cells constituting the tumor tissue. In recent years it has become well recognized that lymphoid cells change their morphology during a division cycle and differentiation. However, unlike the precursors of the myeloid and erythroid cells, the precursors of the cell lines in the lymphoid system in healthy individuals and in diseased individuals often cannot be readily differentiated by simple histologic or cytologic methods.

The histologic classification of Hodgkin's disease takes into account the relative proportions of the different cell types, their arrangement in the node, and the overall effect on the architecture of the node. It has been found valuable in helping to predict prognosis. Most Hodgkin's disease lesions have varying numbers of multinucleate cells, described by Sternberg in 1898 (*1*) and characterized in detail by Reed in 1902 (*2*). Most authorities consider the finding of Reed-Sternberg (R-S) cells a prerequisite for the diagnosis of Hodgkin's disease, though cells resembling R-S cells may be present in conditions other than Hodgkin's disease (*3–5*). It would seem that, because of the diagnostic importance of R-S cells, undue attention has been paid to them in Hodgkin's tissue. The study of the proliferation of cells in Hodgkin's disease is complicated by a mixed population of cells. Some of these cells are indistinguishable morphologically from normal lymphoid cells, though functionally they may be abnormal. Others are grossly abnormal and have all the hallmarks of malignancy. To gain information about cell proliferation in Hodgkin's disease, the various populations must be identified and their proliferative capacity defined. For this purpose a technique of sequential analysis involving cytologic identification, autoradiography, and quantitative cytochemistry was used. It was initially evolved to study proliferation in normal nodes undergoing immune reactions and was later used to examine proliferation in infectious mononucleosis (*6*), Burkitt's tumor (*7*), lymphosarcoma, and reticulum cell sarcoma (*8, 9*). The results of our studies of 10 cases of Hodgkin's disease have been published previously (*10*). Recently, this study has been applied to more cases and has been supported by examination of Hodgkin's tissue labeled by injecton of tritiated thymidine (^{3}H-TDR) in vivo.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

MATERIALS AND METHODS

The sequence of analysis has been detailed elsewhere (*8, 10*). The method used was as follows. Cell suspensions prepared from fresh biopsy material were labeled in vitro with ^{3}H-TDR. A photographic map of a suitable area of a Giemsa-stained smear was prepared; cells were identified according to the cytologic criteria described and marked on the photographic map. The smear was then stained by the Feulgen method, and the DNA content obtained by the measurement of the light absorption at 5700 A. Finally, an autoradiograph was made to identify the cells synthesizing DNA. Other slides were prepared for autoradiography so that the labeling index could be measured with a much larger cell sample.

In vivo labeling.—Since the preparation of cell suspensions probably destroys or damages some cells or fails to extract all the cells from the fibroreticular framework of the node, an in vivo labeling system was derived. In vivo labeling has 2 advantages: 1) The preparation of a cell suspension is no longer necessary, since a simple node imprint is adequate, and 2) labeling can be studied on histologic sections and related not only to cell morphology but also to the histologic architecture and vascularity and, with cryostat sections, the cells can be defined histochemically.

In vivo labeling by direct injection was done on 5 patients undergoing laparotomy and in 2 patients with skin nodules. ^{3}H-TDR, 20 μCi in 0.5 ml sterile saline, was admixed with patent blue and injected directly into an obviously involved node or nodule. At laparotomy, the injected node was left undisturbed for approximately 30 minutes; after removal, the blue-stained areas were cored out and the remainder of the node was retained for routine histologic examination. The portion infiltrated with ^{3}H-TDR was divided; part was fixed in formol-saline for preparation of 4μ sections, and part was fixed for electron microscopy and imprints were prepared.

In one patient with nodular sclerosis (NS), who presented with multiple skin nodules surrounding a large axillary mass, 2 nodules were given simultaneous injections of ^{3}H-TDR. One nodule was removed 30 minutes later and the other after 24 hours.

Identification of cell types.—The appearances of the main cell types in Hodgkin's tissue are illustrated in figures 1–4.

Reticulum cell.—For this investigation, the reticulum cell and the histiocyte were considered synonymous. Electron microscopy confirmed that many of these large dendritic histiocytes were macrophagic. Other macrophages in the nodes were much smaller, and their cytoplasm was in contact with far fewer cells. These smaller macrophages were also found in the thoracic duct lymph in patients with Hodgkin's disease (*11*).

Hodgkin's cells.—In a previous publication (*10*), these Hodgkin's cells were described as "abnormal reticulum cells." These cells were mononuclear. The nuclear chromatin network was open, and large nucleoli which might be multiple were present. The cell cytoplasm was often abundant and frequently vacuolated. Nuclear lobulation was common. Binucleate and multinucleate cells (R-S cells) were morphologically similar to the mononuclear Hodgkin's cells but were less numerous (figs. 1–4).

Transformed lymphocytes.—These large cells were identified by their intensely basophilic cytoplasm and their fine chromatin network which usually stains darker than the Hodgkin's cells. They were morphologically similar to the atypical lymphocytes in infectious mononucleosis and to lymphocytes transformed by phytohemagglutinin (PHA). The cytoplasm was usually less abundant in this cell type than in the Hodgkin's cell and a juxtanuclear, pale-staining Golgi area was often observed. The nucleus was usually rounded or oval, but binucleate cells of this type were rare. Cells showing features intermediate between the Hodgkin's and transformed lymphocyte types were found in most cases. In some node imprints, it was frequently impossible to make such a distinction, and labeling indices of the large cell population as a whole were obtained.

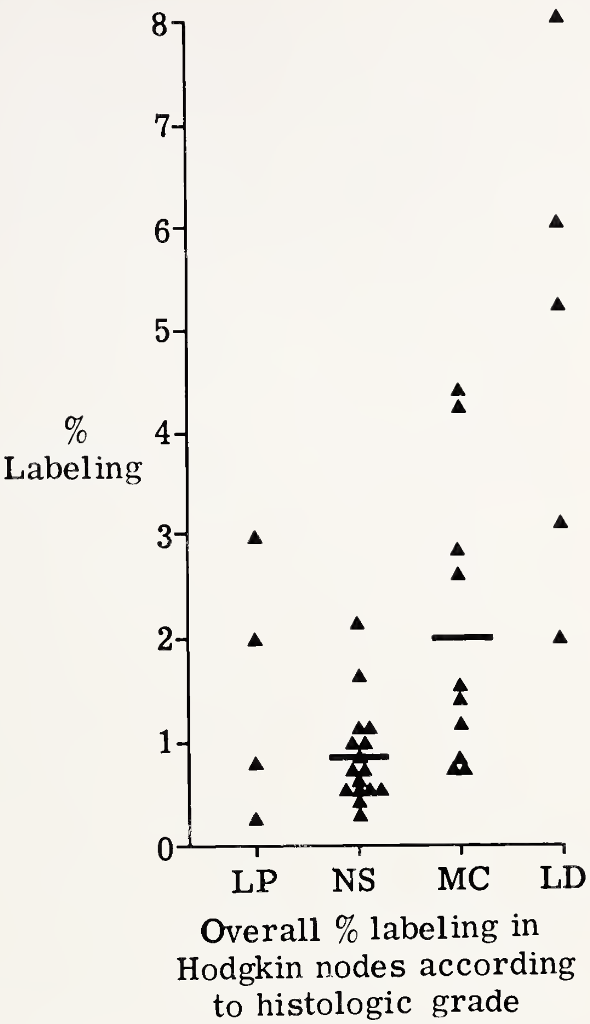
Medium-sized and small proliferating lymphoid cells.—Medium-sized lymphoid cells with basophilic cytoplasm and perinuclear, clear areas were present in most preparations. Sometimes small proliferating cells of the lymphocyte type were identified by their labeling with ^{3}H-TDR. These small microblasts were not suspected to be part of the dividing population until the autoradiographs were examined; although they were usually larger than small lymphocytes, they could not be separated with confidence on morphologic grounds.

RESULTS

In Vitro Labeling With ^{3}H-TDR

Text-figure 1 summarizes the overall in vitro labeling indices in 35 patients according to histologic grade. Labeling varied from 0.2 to 8.0%. This overlapped with the range found in uninvolved lymph nodes from 8 patients undergoing radical mastectomy (0.3–1.1%) and in nodes showing reactive hyperplasia from 3 patients (2.2–9.0%) (Peckham—unpublished data). The mean labeling index for NS was lower than that for mixed cellularity (MC), but histologic grades overlapped considerably. The lower labeling index in NS may have reflected reduced cell separation from the collagenous node matrix. The highest labeling indices were found, as expected, in lymphocytic depletion (LD) where labeling may be intense. Table 1 details the labeling index in the various classes of cells in 10 patients with Hodgkin's disease studied by the in vitro method.

In table 1, reference is made to the contribution of the small and medium lymphoid cells to the labeled population as a whole. Since some of these labeled cells were virtually within the size range of the small lymphocyte, it was impossible to obtain an accurate labeling index. Cells falling into the latter category sometimes accounted for most of the labeled cells. Small, dividing lymphoid cells coexisting with an aneuploid tumor cell population were also found in reticulum cell sarcoma (*9*). The labeling index of cells identified as transformed lymphocytes was high, ranging from 34 to 77%. The percentage of labeled Hodgkin's cells was lower (8.6–34.6%). In Patient 9 (LD, reticular type), most proliferating cells were of the Hodgkin's type, and their labeling index was high (34%). Binucleate or multinucleate cells of the R-S cell type rarely incorporated ^{3}H-TDR in the in vitro studies, but cells of this type are fragile and readily damaged during the preparation of free cell suspensions. No uptake of ^{3}H-TDR was observed in nonspecific esterase-positive reticulum cells or macrophages.



TEXT-FIGURE 1.—Histogram of the overall labeling index in Hodgkin's disease nodes. LP=lymphocyte predominance.

TABLE 1.—*Hodgkin's disease: Analysis of ^{3}H-TDR labeling pattern (10)*

Patient	^{3}H-TDR labeling index (%)			Labeled cells of various classes as % of the total No. of labeled cells		
	Overall	Transformed lymphocytes	Hodgkin's cells	Small–medium lymphoid cells	Transformed lymphocytes	Hodgkin's cells
1	2.1	77.7	8.6	10.1	76.8	13.1
2	1.1	None present	9.0	82.0	None present	18.0
3	2.0	34.0*		56.0	44.0*	
4	4.2	34.0	18.0	80.0	12.6	7.4
5	2.6	38.0*		10.0	90.0*	
6	5.2	58.4	20.0	48.9	20.7	30.4
7	1.6	49.6	14.0	61.0	31.0	8.0
8	0.8	48.0	24.0	25.0	64.0	11.0
9	3.1	67.0	34.6	0	18.0	82.0
10	2.9	66.7	19.2	78.4	18.9	2.7

* Represents the labeling index of large cells; no distinction made between different types.

In Vivo ^{3}H-TDR Labeling

With the direct in vivo labeling methods, cell morphology could be preserved and labeling studied on node imprints (figs. 5, 6). In many preparations it was difficult to identify adequate numbers of R-S cells to estimate percentage labeling, but a few lightly labeled cells of this type were found in most preparations. More detailed analysis was done in 2 patients with MC; it was possible to identify 2,000 Hodgkin's cells and 200 R-S cells on the imprints where good cytologic identification could be achieved. The labeling indices were remarkably similar for both patients (table 2). The labeled R-S cells tended to be binucleate with regular nuclei. Multinucleate cells, especially those with nuclear infolding and large nucleoli, were not labeled. As previously noted, some binucleate cells had regular, rounded or oval, medium-sized nuclei, small nucleoli, and basophilic cytoplasm.

The labeling pattern in the in vivo labeled preparations was otherwise similar to that in the in vitro labeled preparations. Examinations of autoradiographs of histologic material labeled in vivo confirmed that in most cases the predominant proliferating type was a medium-sized lymphoid cell. In the patient from whom a skin nodule was removed 24 hours after injection of ^{3}H-TDR, R-S cells were too infrequent to demonstrate any increase in labeling index, though the overall labeling index increased from 3.9% in the pulse-labeled nodule to 8.4% by 24 hours.

Potential Doubling Time

The dynamics of the cellular traffic through a Hodgkin's disease node is unknown. It is likely

TABLE 2.—*In vivo labeling in Hodgkin's disease*

Histology		% labeling			Labeled small cells as % of total No. labeled cells
		Overall	Large mono-nuclear cells	R-S cells	
A) Labeled nodes					
1	MC	0.8	6	—	20
2	NS	1.0	33	—	89
3	NS	0.7	47	—	43
4	MC	0.6	25*	6.5	21.5
5	MC	1.6	33*	8.5	16.5
B) Labeled skin nodule					
1	LD	14.6			
2(*a*) (Pulse label)	NS	3.9			
(*b*) (Biopsy, 24 hr after labeling)	NS	8.4			

* Hodgkin's cell labeling index.

that, despite marked involvement of the nodes, the traffic of lymphocytes in and out of the node is not grossly impeded and their numbers in the peripheral blood and thoracic duct lymph are related (*11*). Hence it can be assumed that much of the cell production from the lymphoid cells will join the body pool of lymphocytes, though a portion may be retained in the node and contribute to the expansion of the tumor mass. On the other hand, the loss of Hodgkin's and R-S cells from the node is probably small, since they are rare in the blood and sparse in the lymph (*11–13*). In table 3, the potential doubling time of the Hodgkin's cells was calculated according to the method of Steel (*14*) and compared to other forms of lymphoma and Burkitt's tumor. These figures suggest that in all these conditions there was a high death rate in the tumor cell population, for the nodes never expanded at their full potential growth rate.

DNA Content

These measurements were detailed elsewhere (*10*), and they will only be summarized in this paper. In 10 cases of Hodgkin's disease, the small lymphocytes, macrophages, and lymphoblasts had a diploid DNA content. Polyploid lymphoblasts were found occasionally in populations of transformed lymphocytes produced by stimulation with PHA in vitro.

The Hodgkin's cells showed various DNA distribution patterns: In some nodes the modal value was diploid, in others the value was hyperdiploid, and higher modal DNA contents were observed. The general evolution of the modal DNA content toward a hypotetraploid level was similar to that found in many other forms of malignant tumors. Chromosome analyses in our own series (*10*) and those of other workers (*15–18*) confirmed that cells with 46

Table 3.—*Potential doubling time of tumor cell population in human lymphomas*

Tumor	Number of tumors measured	Median labeling index	Potential doubling time in days	References
Hodgkin's disease	10	25.0	1.8	(*10*)
Reticulum cell sarcoma	13	28	1.6	(*9*)
Lymphocytic lymphoma	12	28	1.6	(*9*)
Histiocytic-lymphocytic	5	11.6	4.0	(*9*)
Burkitt's tumor*	26	32	1.4	(*5*)
	23	24.0	1.9	

Calculated according to Steel (*14*): $T = \frac{ts}{LI}$; $\lambda = 0.75$; and $ts = 15$ hours†

* Iversen: Personal communication.

† LI=labeling index; T=generation time; and ts=duration of S phase. Recent evidence by Schiffer (*19*) suggests ts may be shorter and consequently the potential doubling time will be more rapid.

chromosomes were the most common proliferating cells, and smaller aneuploid populations in the near-diploid or near-tetraploid range were also present.

DISCUSSION

The studies made initially with ^{3}H-TDR in vitro and subsequently confirmed by labeling in vivo indicate 2 populations of proliferating cells in Hodgkin's tissue. The lymphoid cells are frequently the predominant proliferating element. However, the labeling indices of the subclasses of lymphocytes are difficult to measure accurately, since it is often uncertain what range of cell sizes should be included under a particular heading. Transformed lymphocytes grow rapidly during their cell cycle; the high labeling indices we have reported for these cells may have been due to an underestimate of the size of the G_1 compartment for this cell type. On the other hand, autoradiography has demonstrated small, labeled lymphoid cells in the nodes that, on morphologic grounds alone, would have been thought unlikely to be members of the proliferating lymphoid cells.

In all patients, the Hodgkin's cells were a proliferating population; most cells had high labeling indices. In our series we tended to include within this group only cells with clear-cut abnormalities of the nucleus and nucleoli. Examination of imprints and electron microscopic preparations indicated there may be smaller histiocytic or reticulum cells that might be part of the Hodgkin's cell group, but these cells were ignored in this study, as their inclusion would introduce even more subjective errors into the investigation. Schiffer (*19*), in his study of cell proliferation of Hodgkin's disease, thought there might be smaller cells in the Hodgkin's cell group that could represent a maturation process akin to that in acute lymphoblastic leukemia. As in cells of leukemia, these smaller cells were not labeled with ^{3}H-TDR.

DNA measurements indicated the bulk of the Hodgkin's cell population is at the G_1 mode, and these cells are probably of 2 types, cycling cells in G_1 and noncyclic cells. It is not known whether, in common with many other forms of cancer, some of these noncyclic cells have the latent capacity for division.

The evolution of the DNA values toward a hypotetraploid mode is typical of many forms of neoplasms. Likewise, the formation of binucleate and multinucleate cells with high DNA contents is seen in several forms of carcinoma. Though weak labeling with ^{3}H-TDR suggests that some DNA synthesis occurs in R-S cells, more probably they arise primarily from acytokinesis of mononuclear Hodgkin's cells. The multinucleate variants could come from nuclear segmentation of polyploid mononuclear precursor cells or by cell fusion.

An attempt to demonstrate the passage of label from the mononuclear Hodgkin's cell pool to the multinucleate R-S cells in one patient with multiple skin nodules was unsuccessful because of the paucity of the latter cell type. This technique has been used by Nameroff et al. (*20*) to investigate differentiation in a skeletal muscle tumor where the multinucleated nonproliferating, striated cells were shown to arise from labeled, mononuclear, nonstriated cells. Whatever their mode of production, it seems improbable that R-S cells contribute to the expansion of the tumor. The presence of these cells in large numbers often indicates a rapidly expanding tumor in which Hodgkin's cells may be the main cell element in the tumor. There is little information on the cell kinetics of human lymphoid tumors. In a recent study, the generation time of tumor cells in a patient with reticulum cell sarcoma was estimated to be 54 hours (*21*). This is similar to values reported for a variety of other human tumors; if the generation time of either proliferating cell population in Hodgkin's disease is of this order, clearly the potential doubling time greatly exceeds the observed rate of expansion of the tumor, as is the case for many other forms of lymphoma. At their fastest rate of growth, small nodules of Burkitt's tumor can double their size in about 4 days. This growth rate declines as the mass grows, but the labeling index remains high (Iversen, personal communication). Charbit et al. (*22*), reviewing the evidence from the literature on the observed growth rate of 51 lymphomas, including 15 cases of Hodgkin's disease, reported that the mean doubling time of these tumors was 29 days with a 95% confidence interval of 23–37 days. In part, this immense discrepancy between the potential doubling time and the true rate of tumor increase can be explained by the fact that the tumor cells in Hodgkin's disease and several other lymphomas form only a part of the total cell population in the lesion, but cell death is liable to be a major factor influencing the expansion of the tumor cell component of the lesion.

The fact that, in biopsies of Hodgkin's tissue done 24 hours after pulse labeling, the labeling index had more than doubled is consistent with a high proliferative rate of populations of both lymphoid and Hodgkin's cells. The lymphoid element appears to have opportunities to leave the lesion, but the Hodgin's cells tend to stay within the lesion.

Clearly Hodgkin's disease involves highly complex local changes in the node as well as alteration of the function of the lymphoid cells. Study of the proliferation of these cells has given some limited information on one facet of the organization of this tumor.

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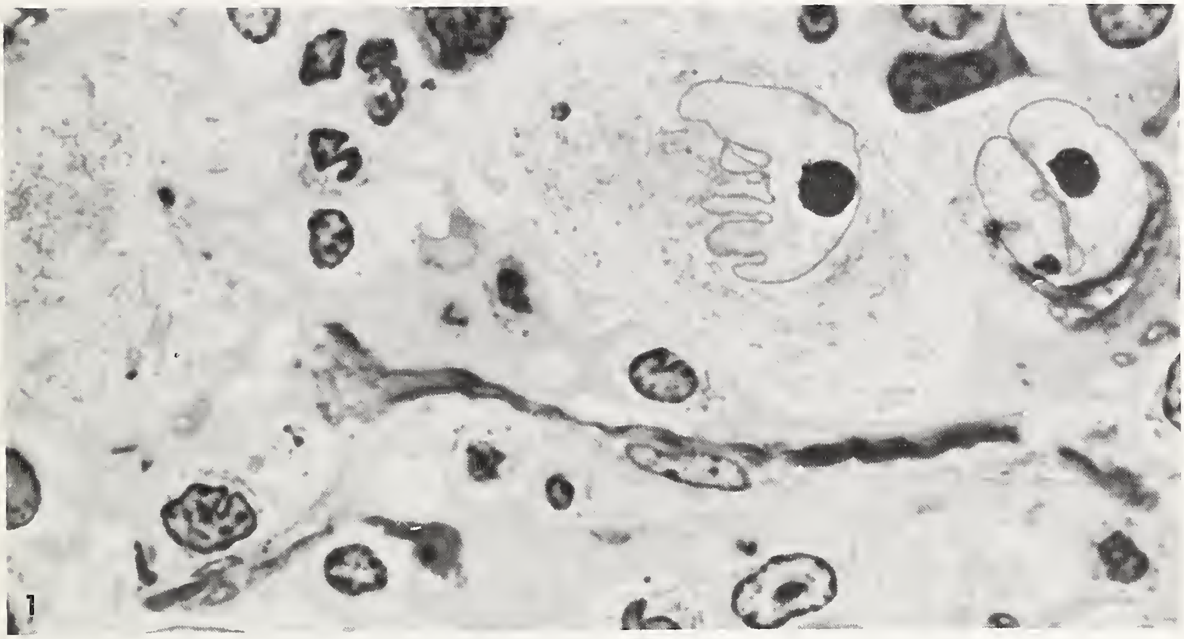


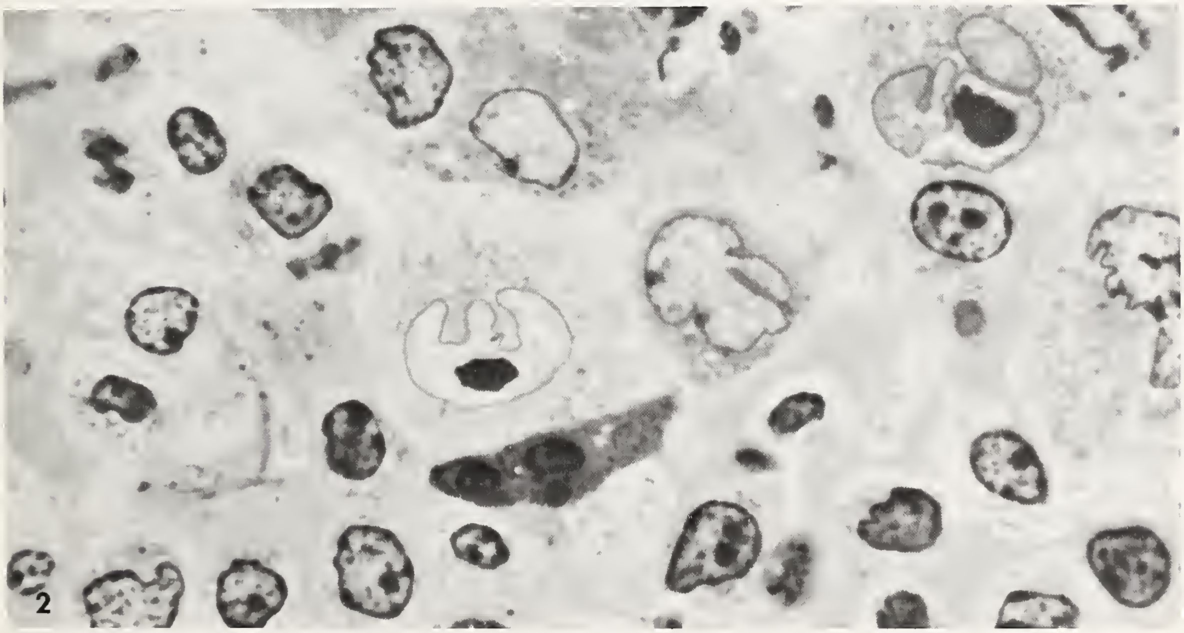
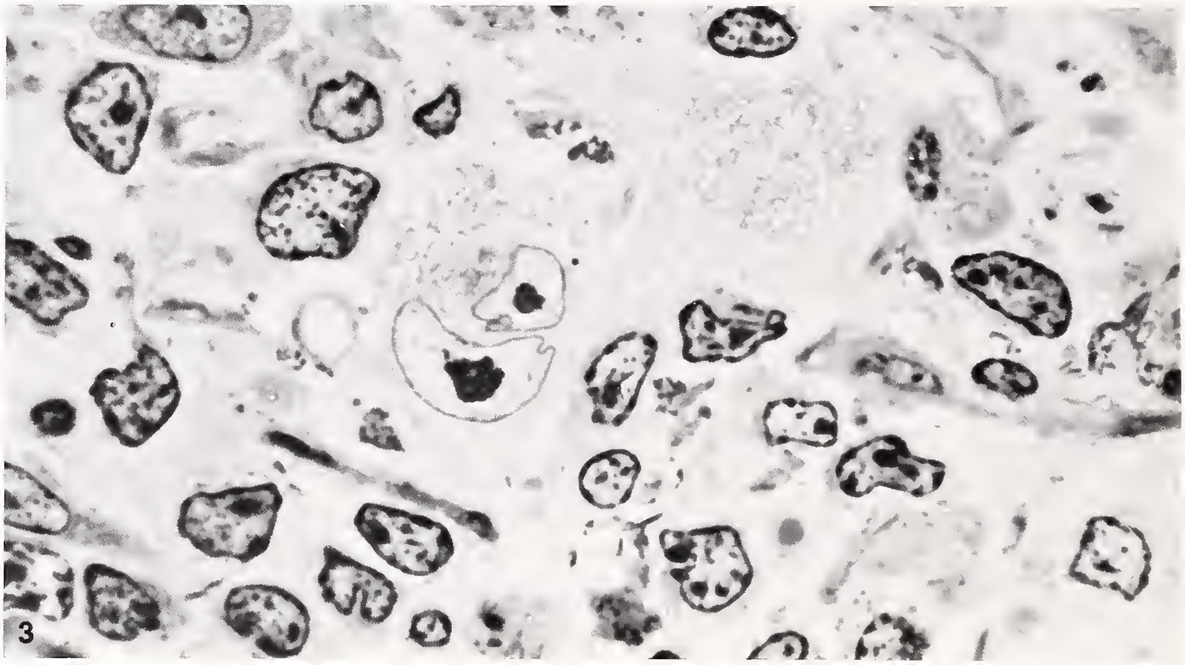


Figure 1.—Hodgkin's cells showing indented nucleus and large, dense nucleolus. Cytoplasm contains fine strands of endoplasmic reticulum. Toluidine blue. × 1100

Figure 2.—Hodgkin's cell with eosinophil (dark-staining cell) in close contact. Toluidine blue. × 1100



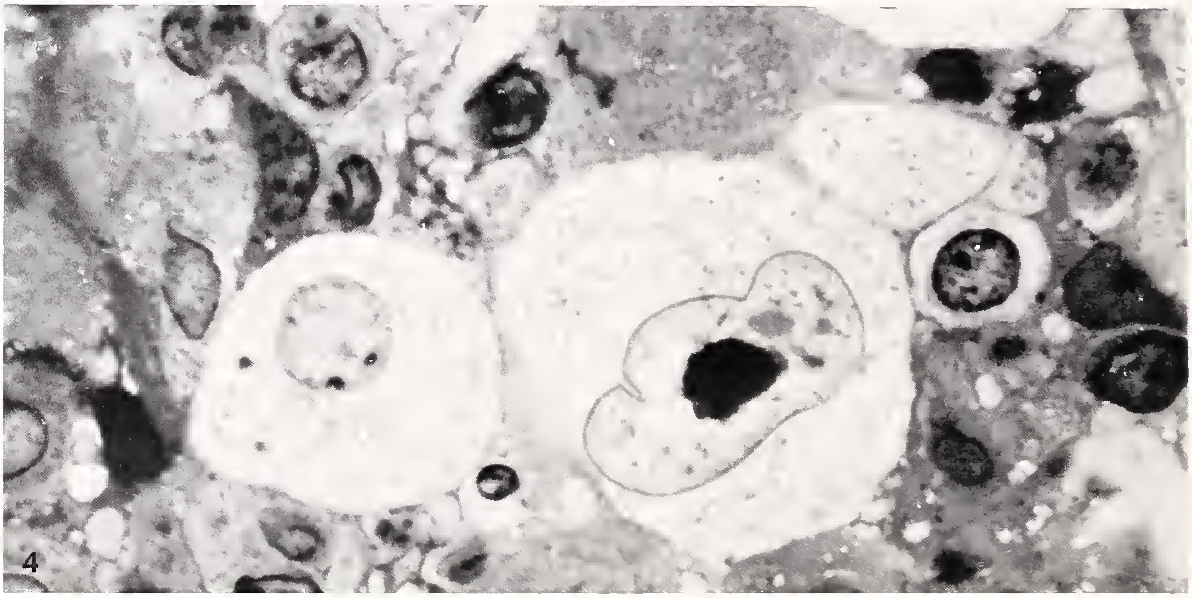
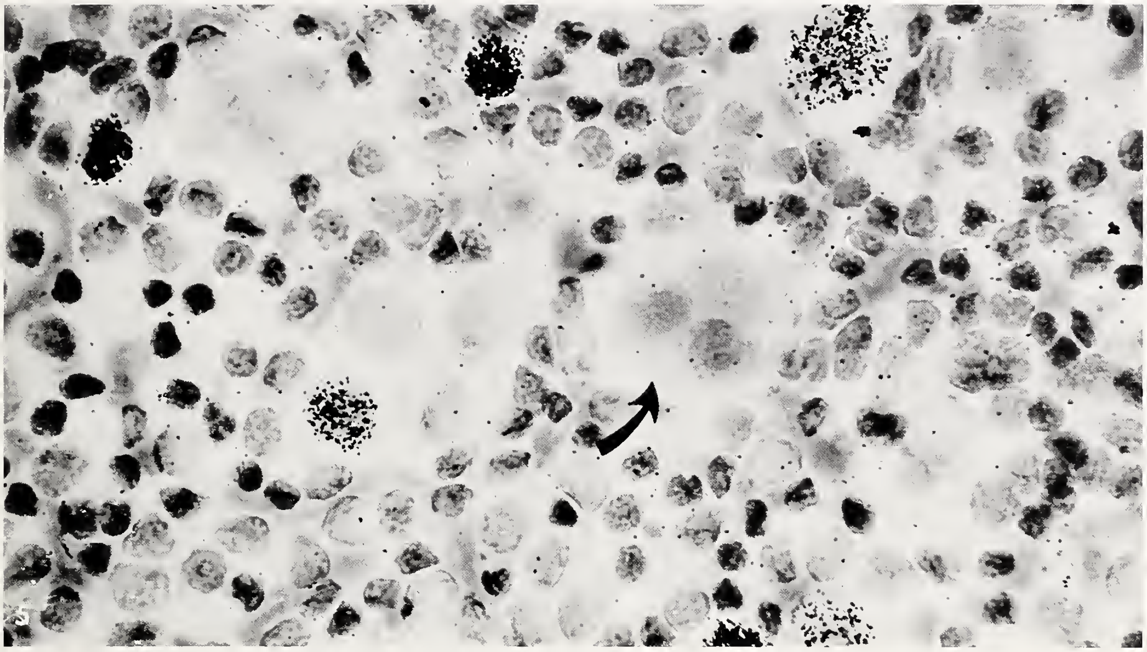


Figure 3.—Small R-S cell surrounded by small lymphocytes. Toluidine blue. × 1100

Figure 4.—Hodgkin's cells with pale-staining cytoplasm, showing typical, well-delineated nuclear membrane and paucity of condensed chromatin. Toluidine blue. × 1200



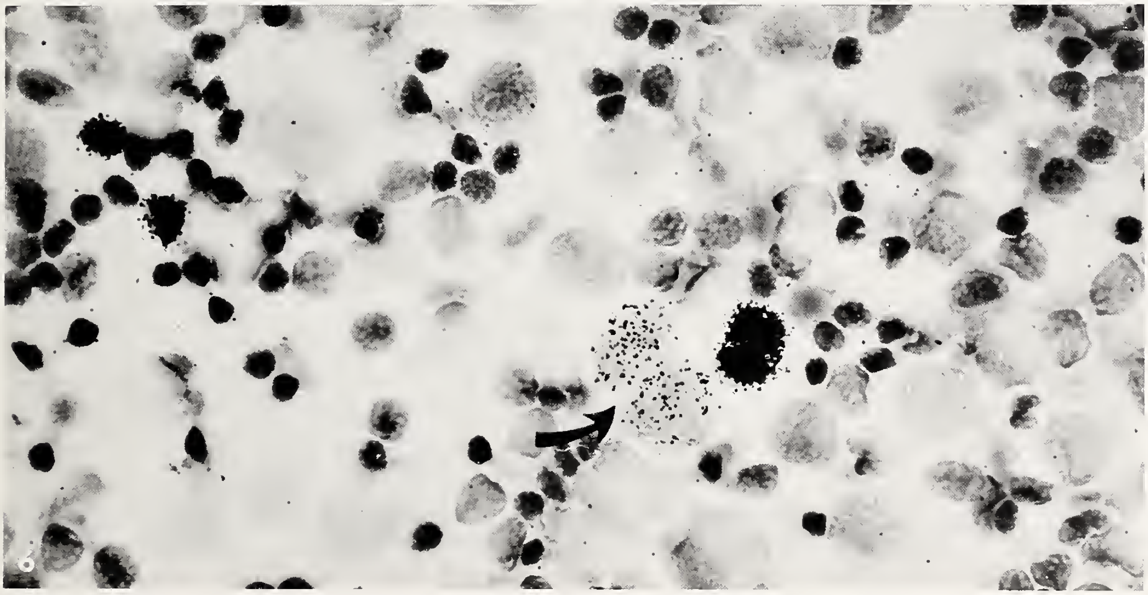


FIGURE 5.—Imprint of in vivo labeled Hodgkin's disease node showing unlabeled R-S cells (*arrow*). Hematoxylin. × 480

FIGURE 6.—Imprint of in vivo labeled Hodgkin's disease node showing weakly labeled R-S cell (*arrow*). Hematoxylin. × 480

Invited Discussion: Proliferation of Cells in Hodgkin's Disease[1]

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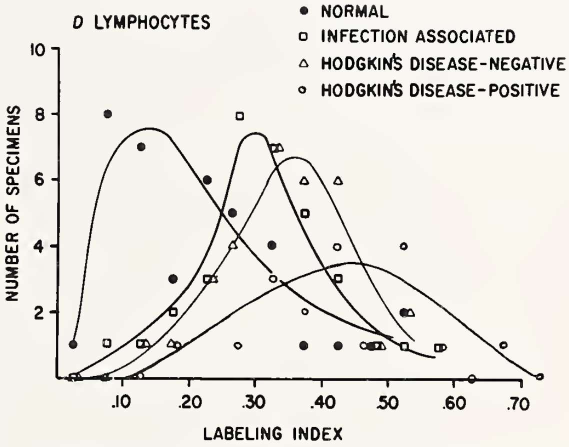
MY STUDIES on the proliferative capabilities of the Reed-Sternberg cell are considerably more limited than those of Peckham (*1*), my interests being focused on the lymphocytes found in Hodgkin's disease as well as those found in normal individuals.

Briefly, of 12 patients with Hodgkin's disease coming to laparotomy, half had Reed-Sternberg cells; of these, most had some labeled cells. Labeling with ^{3}H-thymidine, however, varied from only occasional to >30%. In the latter specimen, enough labeled cells could be found to estimate, albeit in a statistically poor way, the DNA synthesis time by an in vivo-in vitro double-labeling technique. The time was found to be 13.1 hours. If we assume the growth fraction is 1, the cell cycle time would be about 40 hours. If the growth fraction is <1, as is most probable, the cell cycle time would be shorter.

In regard to the proliferative capabilities of lymphocytes, I have divided lymphocytes from solid tissue into 4 classes, depending on their relative size and staining characteristics. Data indicate that this is, morphologically speaking, a valid proposition—functionally it is probably quite crude. The large, basophilic lymphoid cell, which I call a *D* cell, is distinguishable from others not only by morphology but by being in a population of cells with thymidine-labeling indices that are normally distributed (text-fig. 1). For this, and other reasons, I believe that this cell has a growth fraction of 1 and that estimates for cell cycle times are valid. The *A* cell or small, nonbasophilic lymphocyte does not label significantly, but the small, basophilic cell (the *B*) and the large, nonbasophilic cell (the *C*) label quite well, but do not have similar distributions. This indicates there is more than 1 population of each of these cells. One guess might be that there is a distinct growth fraction associated with these cell types.



TEXT-FIGURE 1.—*D* cells with thymidine indices that are normally distributed.

The percentage distribution of the various lymphoid classes was determined in >150 samples of lymphoid tissue and is shown in table 1. Infection-associated lymphoid tissue includes tonsils, adenoids, and lymph nodes draining an acute infection. The lymphoid hyperplasia nodes were removed surgically because of unexplained sudden growth. The Hodgkin's-negative nodes were from patients with Hodgkin's disease, but these nodes were not diagnostic, whereas the Hodgkin's-positive nodes contained Reed-Sternberg cells.

Although the infection-associated and benign lymphoid-hyperplasia groups are all significantly

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

Table 1.—*Percentage of lymphoid cells in subpopulations**

Cell	Normal	Infection-associated	Lymphoid hyperplasia	Hodgkin's disease	
				Negative	Positive
A	92.8(0.6)	83.4(1.2)†	86.1(2.7)†	94.0(0.7)	92.4(0.8)
B	4.8(0.5)	10.0(0.8)†	9.1(2.1)†	3.3(0.4)†	4.1(0.4)
C	1.8(0.1)	3.7(0.3)†	3.0(0.4)†	2.1(0.2)	2.8(0.3)†
D	0.6(0.1)	2.9(0.3)†	1.8(0.3)†	0.6(0.1)	0.7(0.1)

* Numbers in parentheses=1 SE of the mean.
† Significantly different than normal, $P<0.01$.

different from normal, the Hodgkin's disease groups are not usually different from normal. However, the thymidine-labeling index of these cells (table 2) shows significant increases in the Hodgkin's disease groups, though not necessarily to the extent of the infection and hyperplasia groups. To be even more detailed and precise, we can examine the labeling index according to the site of origin of the lymphocytes in question (table 3). Here, Hodgkin's disease-positive specimens clearly label more vigorously than the Hodgkin's disease-negative specimens, which, in turn, label more than normal.

Table 2.—*Variation of thymidine-labeling index in subpopulations**

Cell	Normal	Infection-associated	Lymphoid hyperplasia	Hodgkin's disease	
				Negative	Positive
A	0.0001(—)	0.0013(.0002)†	0.0009(.0003)†	0.0002(—)†	0.0006(.0001)†
B	.040(.006)	.196(.018)†	.166(.020)†	.096(.015)†	.178(.025)†
C	.061(.007)	.191(.012)†	.279(.014)†	.100(.014)†	.155(.023)†
D	.212(.021)	.318(.018)†	.500(.030)†	.342(.017)†	.409(.031)†
Total	.004(.001)	.039(.004)†	.030(.003)†	.006(.001)†	.012(.001)†

* Numbers in parentheses=1 SE of the mean.
† Significantly different than normal, $P<0.01$.

In addition to these data, the DNA synthesis times of lymphocytes were determined by in vivo-in vitro double labeling (*2*). As an example of the cytokinetics derived from this and other information, the results for the *D* lymphocytes are shown in table 4. Although the DNA synthesis times are slightly longer than normal in Hodgkin's disease, the cell cycle times are the same or shorter, and the G_1 distribution appears less skewed. Putting all this cytokinetic information together for production-rate determinations, we get the information in table 5.

Line 1 for each cell type represents the actual number of lymphocytes born in each cell class related to the normal. Line 2 represents the normalized fractional production rate or reciprocal of the turnover time. In the *A* class of cells, while there is a general increase in infection, hyperplasia, and Hodgkin's disease, the normalized actual production rate equals the normalized fractional production rate, as one would expect in a steady state. In the *B, C,* and *D* cell classes, the infection and hyperplasia actual production rates greatly exceed the fractional production rate. If we assume no great change in the total lymphocyte population—manifested by an increase in size of the lymph node—this then

TABLE 3.—*Variation of thymidine-labeling index by site**

Cell	Spleen			Para-aortic and iliac			Mesenteric		
	Normal	Hodgkin's disease		Normal	Hodgkin's disease		Normal	Hodgkin's disease	
		Negative	Positive		Negative	Positive		Negative	Positive
A	0.0001(—)	0.0003(.0001)†	0.0006(.0001)†	0.0001(.0001)	0.0002(—)	0.0008(.0002)†	0.0001(—)	0.0001	0.0008
B	.037(.009)	.073(.013)†	.151(.018)†	.028(.003)	.127(.026)†	.262(.078)†	.057(.019)	.046	.112
C	.046(.008)	.061(.009)	.113(.016)†	.049(.011)	.137(.024)†	.257(.063)†	.069(.024)	.109	.122
D	.186(.041)	.321(.028)†	.377(.036)†	.282(.068)	.373(.020)†	.508(.080)†	.252(.053)	.326	.342
Total	.003(—)	.007(.001)†	.011(.001)†	.005(.001)	.006(.001)	.017(.004)†	.003(.001)	.003	.008

* Numbers in parentheses=1 SE of the mean.
† Significantly different from normal, $P<0.01$.

TABLE 4.—*Cytokinetics of D lymphocytes**

	Normal	Infection-associated	Lymphoid hyperplasia	Hodgkin's disease	
				Negative	Positive
Mean LI	0.212(.021)	0.318(.018)	0.500(.030)	0.342(.017)	0.409(.031)
Mean TS(hr)	8.4(0.3)	8.4(0.3)	8.4(0.3)	9.0(0.7)	11.2(0.7)
Mean TC (hr)	39.6(4.2)	26.4(1.8)	16.8(1.2)	26.3(2.4)	27.4(2.7)
Maximum TC(hr)	336	67	21	120	64
Minimum TC(hr)	16.8	16.0	13.1	15.7	15.4

* Number in parentheses = 1 SE of the mean. LI = thymidine labeling index; TS = DNA synthesis time; and TC = cell cycle time.

TABLE 5.—*Production of lymphocytes**

		Normal	Infection-associated	Lymphoid hyper-plasia	Hodgkin's disease	
					Negative	Positive
A	1. Number born/lymphocyte/hr$\times 10^3$* normalized to 1	1	11.9	8.6	2.4	6.1
	2. Fractional production rate/hr$\times 10^3$† normalized to 1	1	12.8	9.1	2.3	6.1
B	1. Number born/lymphocyte/hr$\times 10^3$* normalized to 1	1	10.2	7.4	1.6	3.5
	2. Fractional production/hr$\times 10^3$† normalized to 1	1	4.9	4.2	2.3	4.1
C	1. Number born/lymphocyte/hr$\times 10^3$* normalized to 1	1	6.4	7.6	1.7	2.8
	2. Fractional production rate/hr$\times 10^3$† normalized to 1	1	3.2	4.5	1.5	1.8
D	1. Number born/lymphocyte/hr$\times 10^3$* normalized to 1	1	7.2	6.9	1.3	1.7
	2. Fractional production rate/hr$\times 10^3$† normalized to 1	1	1.5	2.4	1.5	1.5

* Derived from fraction of lymphocytes × LI ÷ TS.
† Reciprocal of cell cycle time.

indicates a large cell loss in the proliferative compartment. This loss could be by cell death, migration, or transformation to another class. Probably all these factors can apply, as can be recalled by the findings of labeled tingible bodies in germinal centers, increase in labeled cells in thoracic duct lymph, and the formation of plasma cells following antigenic stimulation.

This great loss of cells is not characteristic of the Hodgkin's disease group seen in the last columns. In fact, the point that Hodgkin's lymphocytes do not behave in this manner may be the cytokinetic indicator that these cells are not responding to an antigenic stimulus—or are responding abnormally.

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SUMMARY OF INFORMAL DISCUSSION ON DNA SYNTHESIS AND CELL PROLIFERATION

R. F. Dorfman asked Doctor Peckham a question about terminology. He had used terms such as "lymphoid cell," "reticulum cell," and "Reed-Sternberg cell," suggesting that they may be different cells and may have a different source of derivation. Yet, he had shown some labeling of the nuclei of Reed-Sternberg cells. Did he feel that the cell that he was calling a reticulum cell is different from the transformed lymphocyte and the Reed-Sternberg cell? **M. J. Peckham** replied that these are simply names for cells whose morphology is different. The names do not imply whether they are different or related. Certainly cell populations can show differences in morphology simply due to the passage of the cells through the cycle. The cell gets larger and becomes more basophilic due to increasing amounts of RNA. He would regard the Reed-Sternberg cell as the same as an abnormal reticulum cell or Hodgkin's cell, but a multinucleate variant of it. It seems reasonable to regard them as part of the same family of cells, in which the Hodgkin's cell is the mononuclear equivalent of the Reed-Sternberg cell.

Peckham then asked Doctor Schiffer whether, when he says that 30% of Reed-Sternberg cells were labeled, he means mainly mononuclear cells and not multinuclear cells. **L. M. Schiffer** replied that 30% of multinuclear cells were labeled.

H. S. Kaplan asked Doctor Peckham whether it would be possible, with the technique he and Doctor Schiffer are using, to stimulate the preparations with a mitogen such as phytohemagglutinin (PHA) or pokeweed mitogen and then to compare the labeling indices of known classes of cells before and after stimulation. There is an extremely intriguing case reported by Sinks and Clein some years ago in which 93% of the cells in the peripheral blood were Reed-Sternberg cells. There was a true Reed-Sternberg cell leukemia. They looked at the PHA response of those peripheral blood cells and found that the cells did respond to PHA. If so, this would be rather intriguing evidence that the Reed-Sternberg cells may indeed be derived from immunocompetent lymphoid cells. However, we do not encounter Reed-Sternberg cell leukemias very often. It would, therefore, be of great interest if they could adapt their techniques to this kind of investigation.

Peckham said he would like to have seen the cells from the Sinks and Clein paper, because the illustrations had not convinced him that these were Reed-Sternberg cells. The cells were rather round and regular with basophilic cytoplasm. However, the patient certainly did have Hodgkin's disease, and the cells did show a moderate increase in labeling index with PHA. What one would really like to do is to be able to separate Hodgkin's node preparations into different cell fractions. There are so many different cell types in the crude preparations that just to stimulate the whole lot with a mitogen would yield results which would be very difficult indeed to interpret.

M. Tubiana asked whether there was any difference in the frequency of aneuploidy in the different histologic types of Hodgkin's disease.

Peckham answered that he and his colleagues had not really studied enough patients to make a meaningful correlation with histology. However, they had done some cases of each histologic category and had found aneuploidy in all of them, including lymphocytic predominance. Thus, Hodgkin's disease by this criterion is a malignant tumor, even in its least aggressive histologic form. However, the labeling index is much higher in the lymphocytic-deficient type where the growth rate is more rapid and one sees more cells of Hodgkin's type.

Chromosome Changes in Hodgkin's Disease [1]

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SUMMARY—Chromosome abnormalities are common in preparations of Hodgkin's disease tissue as they are in human cancers in general. Such abnormalities may in part represent one or more stages of a progressive series of changes such as: 2n (or near-2n) → 4n (or near-4n → etc.). The high rate of chromosome mutation in this and other cancers remains unaccounted for; but in a given tumor the end result, cytogenetically, most often represents selection of one of the karyotype variants.—Natl Cancer Inst Monogr 36: 197–201, 1973.

CHROMOSOME ANALYSIS in Hodgkin's disease largely supports the concept that the disease is neoplastic (*1–11*). In most human solid cancers, the chromosome complements are grossly aneuploid, usually with a marked excess of chromosomes (50 to >100) and often with abnormal so-called "marker" chromosomes. Changes in benign human cells never match the degree of abnormality often seen in cancer cells. Chromosome changes in congenital abnormality (Downs' syndrome, Klinefelter's syndrome, etc.) rarely involve more than 1 chromosome, though, on occasion, 2 or more sex chromosomes may be in excess and rare cases of balanced triploidy (3nXXY or 3nXXX) have been reported. About the only significant exceptions to the above rule are meningiomas. These tumors often also are characterized by gross chromosome abnormality, though, of course, in some cases, these may in fact be malignant tumors. In Hodgkin's disease, at least 20 of about 38 reported cases had cells with counts ranging from 51–154 chromosomes (table 1). Often these cells contained marker chromosomes or various other aberrations.

In addition to the population of grossly aneuploid cells, there is a second population of diploid or near-diploid cells. Often this population exceeds in number the population of aneuploid cells. Frequently these cells appear classically diploid. In other instances, variations from diploidy might well be due to counting error or similar problems. However, there remain a few cases, perhaps 4 or 5, in which the cells are pseudo-diploid (i.e., with markers or imbalances) or hyperdiploid (48–50 chromosomes); these abnormalities occur considerably more frequently than would be expected in counts and karyotypes of completely normal lymphocytes (*2, 4, 5, 7, 8*). In addition, these cells are found growing in suspension in cultures not treated with phytohemagglutinin (PHA). Such growth does not usually occur with benign lymphocytes.

One possible explanation for the finding of 2 cell populations is that one consists of cancer cells and the other of benign-reacting cells, presumably lymphocytes. Classically diploid cells are seen in addition to aneuploid cells when preparations are made from cancers metastatic in lymph nodes (*12, 13*). They may also be found in primary cancers and sometimes can be correlated with lymphocytic infiltrates seen in histologic sections of tumors. This may account for some of the classically diploid cells seen. However, as mentioned (*see* also table 1), sometimes the cells seen are pseudo-diploid or hyperdiploid

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

Table 1.—*Summary of chromosome findings in Hodgkin's disease**

Reference	Case	Chromosome Nos. or modes ( )		Remarks
		2n or near	Polyploid	
(1)	—	2n	79–90 (83)	2 markers in a cell with 82 chromosomes.
(2)	—	46, 47	near-4n	Marker seen in near-2n cells occurred twice in near-4n cells.
(3)	—	—	79–83 (83)	—
(4)	3	46	70–75	—
(4)	4	46, 48, 49	—	—
(5)	8	46, 47, 49 50 (46)	—	—
(5)	9	46, 47, 48	92	Said to be deleted short arms on E chromosomes.
(5)	10	46	69–76	Paragranuloma.
(6)	—	43–50 (46, 48)	—	Peripheral blood cultures.
(7)	1	45–47; pseudo-2n	86	Pseudo-2n cells had various markers. Cell with 86 had 6 markers, 4 of them very similar. A number of cases in *(7)* were said to have attenuated secondary constrictions especially on chromosome 9.
(7)	2	46	69–139	Polyploid cells had multiple markers which varied from cell to cell. One cell was pulverized.
(7)	3	46	—	—
(7)	4	46	63–75	One near-3n cell had 2 very similar ring chromosomes. Different markers in other cells.
(7)	5	46	85–91 (85)	Hodgkin's disease sarcoma. Same marker occurred in several polyploid cells.
(7)	6	—	near-4n	—
(8)	1	46	69–70	—
(8)	2	46	71–84 (84)	—
(8)	3	46	—	—
(8)	4	46; pseudo-2n	73, 83	One 2n cell had various aberrations.
(8)	5	48	—	Paragranuloma.
(8)	7	46	77–86 (81)	Polyploid cells had same 2 markers in most cells.
(8)	8	46	73	Various abnormalities in both diploid and polyploid cells.
(9)	13	46	—	—
(9)	14	46; pseudo-2n	64–80	Numerous minute chromosomes in polyploid cells.
(10)	17	46, 47, 49, 50	—	Some minute and marker chromosomes.
(10)	18	46; 46+ pseudo-2n	—	Deleted short arms on E group chromosomes in cases 18 and 19.
(10)	19	46	69–76	Paragranuloma.
(10)	20	46	51–100	Chromosomes in polyploid cells often were "frayed or unraveled" (pulverization).
(10)	21	46	—	—
(11)	10–13	46	near-4n	Counts of polyploid cells in illustrations are 72, 123, 134, and about 154.
(11)	14	46	—	—
(11)	15	46–48	near-4n	Cases 15–20 had "histologic patterns suggestive of, but atypical for Hodgkin's disease."
(11)	16	46–49	—	—
(11)	17	46, 48	—	—
(11)	18	46–49	near-4n	—
(11)	19	46–48	near-4n	—
(11)	20	46–47, 50	near-4n	—

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* All of these cases were Hodgkin's disease granuloma unless otherwise noted. In series where tissue cultures were done, none exceeded 3 days and at least some in each case were done without phytohemagglutinin. Apparently only 4 of these cases (*2, 6, 11*) had been treated prior to chromosome analysis.

or have distinct marker chromosomes. Moreover, diploid cells are found in cultures without the stimulus of PHA. The following hypothesis may in part account for the chromosome changes in Hodgkin's disease.

Let us assume that, with the possible exception of chronic myeloid leukemia, the chromosome pattern is normal at the earliest stage of oncogenesis, or at the instant of oncogenic transformation. (This does not of course preclude a change, such as a point mutation or a lyzogenization, below the level of resolution available to the classical cytogeneticist.) At some later point, however, most human cancer cells tend to gain or lose chromosomes, and breaks and rearrangements occur. Whereas many of these changes may be lethal for the affected cell, some apparently have a selective advantage. These cells may give rise to clones which come to dominate or replace the original population of cells. Another common event is precise doubling of the chromosome complement. The doubled cells apparently have a strong selective advantage, since this event appears to have occurred in most human cancers, especially those of epithelial origin. However, after doubling, the tendency is to lose chromosomes, and most human tumors are found to have stemlines ranging from about 60 to 80 chromosomes. A schematic outline of this hypothesis is as follows:

$$\begin{tikzcd}[row sep=small] & \text{4n (92)} \arrow[dr] & & \\ \text{2n (46)} \arrow[ur] \arrow[dr] & & \text{near-4n, etc.} \arrow[r] & \text{sub-4n (very variable, occasionally 92+).} \\ & \text{near-2n, pseudo 2-n} \arrow[ur] & & \end{tikzcd}$$

The doubling event may occur repeatedly: near-2n → near-4n → near-8n → near-16n; but the higher numbers usually occur only sporadically and rarely become dominant.

A few tumors either do not show such changes or may become arrested at some point in the progression, and all cells will have the same karyotype (chromosome pattern), near-2n, sub-4n, etc.

If this hypothesis is correct, the results of chromosome analysis will depend partly on the age of the tumor. If we could sample a very early stage, we would expect all the cells to be normal. At a later stage, we might find a mixed population of cells, 2n and 4n or near-2n and near-4n. At the latest stage, we might find only grossly aneuploid cells. Also, if a tumor tends to undergo stages of histologic progression, we might expect to find some correlation with cytogenetic progression.

A possible example of such sequence is found in uterine cervical neoplasia. Although there are differences of opinion, most reports are consistent with some such scheme as:

2n → 4n, near-4n → sub-4n
mild dysplasia (atypia) → moderate dysplasia → severe dysplasia

→ sub-4n → sub-4n
carcinoma in situ → invasive cancer

However, various investigators might shift the upper line right or left with respect to the lower. The shift may be, in part, due to differing criteria for the diagnosis of dysplasia, and, in part, due to the possibility that the correlation between cytogenetic and histologic progression is, in fact, not a very close one. The direction is the same in every case, but, in a particular tumor, one progression may precede or follow the other.

A similar scheme for Hodgkin's disease might be:

2n (as yet unidentified earliest histologic stage) → 2n, near-2n lymphocyte predominance, paragranuloma → near-2n and near-4n or sub-4n nodular sclerosis; Hodgkin's disease granuloma

→ sub-4n
mixed cellularity, lymphocyte depletion; Hodgkin's disease sarcoma

Of course, the nature and incidence of histologic progression are much more problematic in Hodgkin's disease than in cervical neoplasia. However, the chromosome data at least are consistent with progressive cytogenetic change. That a doubling occurs is shown by the many instances of near-tetraploid cells. Often these cells can be shown to have the expected tetraploid number for many chromosome groups (*7*) while, at the same time, will have distinct chromosome abnormalities. What appears to be the same marker chromosome may occur twice in the polyploid cells (*2, 7*) and only once in near-diploid cells (*2*). Atkin and Baker (*19*) kindly permitted me to refer to an unpublished case in which "... 2 different cell-lines were ... found in lymph nodes removed on two different occasions. The first, a supraclavicular node, showed ... (a) ... hyperdiploid cell-line whilst a para-aortic and splenic hilar node removed 3 months later with no intervening treatment showed ... (a) ... hypertetraploid cell line. The karotypes of both cell-lines show a similar chromosomal distribution including the presence of 2 different marker chromosomes in the near-diploid line which are duplicated in the near-tetraploid line."

That cytogenetically abnormal cells give rise to clones is evidenced by finding the same or very similar abnormal karyotypes in many cells. (Note that, on this hypothesis, the finding of clones does not indicate that the tumor arose from an original single cell although such origin is not precluded.) That raw material for selection is still being provided is evidenced by what is often an abundance of distinctly different karyotypes from cell to cell.

Some of the diploid (or near-diploid) cells seen may represent benign lymphocytes. However, at least those which show markers, have chromosomal imbalances (some groups having extra, others missing chromosomes), or are distinctly hyperdiploid (48–50) may well be cancer cells.

The fact that Hodgkin's disease so often shows 2 populations of presumably neoplastic cells may indicate that the disease tends to be at a relatively earlier stage of cytogenetic progression than are most epithelial cancers. This is also suggested by the fact that the polyploid cells are often very close to precise tetraploidy.

Most authors have assumed that such polyploid cells were dividing Reed-Sternberg cells. A possible progression might be:

2n →	near-2n →	near-4n, sub-4n
hypothetical lymphocyte precursor	Hodgkin's cell	Reed-Sternberg cell

or, assuming perhaps that Reed-Sternberg cells are incapable of further division:

near-2n →	near-4n, sub-4n →	sporadic near-8n near-16n, etc.
hypothetical lymphocyte precursor	Hodgkin's cell	Reed-Sternberg cell

It may suffice for the purpose of this discussion to show how Hodgkin's disease cytogenetically resembles other human cancers. The driving force behind the sporadic occurrence of chromosome abnormalities in cancer cells is a deeper problem. There can now be little doubt that benign human cells, even in tissue culture, do not show the high incidence of chromosome abnormality observed in many cancers. As yet, however, there are but meager clues as to the fundamental cytogenetic derangement in cancer cells. It has been observed that accentuated (attenuated) secondary constrictions of chromosomes are common in lymphomas including Hodgkin's disease (*7*). (They may also occur in other types of tumors.) Such constrictions are also common in permanent lymphocyte lines (transformed by Epstein-Barr virus?) (*14*), in simian virus 40-transformed human fibroblasts (*15*), and in lymphocytes treated with herpes simplex (*16*), especially the type II strain possibly implicated in cervical cancer (*17*). The sites of these constrictions tend to be associated with blocks of late-replicating DNA (heterochromatin), and there is some correlation between the chromosomes most affected by spontaneous change and the amounts or concentrations of late-replicating DNA they contain (those with the greatest amounts of heterochromatin tending more often to be lost or perhaps restructured) (*18*). Perhaps these clues will turn out to be mere coincidence, or perhaps they reflect a side effect of some final common pathway in oncogenesis.

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Invited Discussion: Chromosome Studies of Hodgkin's Disease [1,2]

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MILES (*1*) presented a hypothesis for chromosome changes in neoplasia which explains the karyotypic variability of aneuploid cells in tissues involved with Hodgkin's disease. This variability, a striking feature of various forms of neoplastic cells in vivo (*2, 3*), was demonstrated in the Hodgkin's disease material by direct preparations and short-term cultures (*4–10*). However, analyses of long-term cultures obtained from similar Hodgkin's tissues revealed only the presence of cells with approximately diploid chromosome complements (*11–14*). Apparently the in vitro conditions did not favor proliferation of the aneuploid cells.

I present the results of an additional chromosome study performed in conjunction with the long-term cultivation of cells obtained from splenic tissue involved with Hodgkin's disease. The procedures used and types of cells in the cultures are described in a following paper (*15*). To date, chromosome analyses have been performed on 6 cultures sampled 2–3 days after initiation and again after 2–4 months in vitro.

There was a low percentage of dividing cells in the initial material, as reported by others. To offset this difficulty, a large volume of cells was collected for each initial sample. For both sampling times, total populations of cells were collected and processed in the routine manner used for peripheral blood, but no phytohemagglutinin was used. The chromosomes of 50 well-spread metaphases were counted for each preparation, with the exception of the initial sample of one culture in which only half that number of metaphasic cells were observed from an equal volume of cells.

Chromosome enumerations of the 6 cultures are summarized in table 1. Over 50% of the cells were aneuploid in each initial sample; however, the mode for all 6 was the diploid number of chromosomes. This is consistent with the findings of previous studies, with autoradiographic determinations of the extent of DNA synthesis at this time (*15*), and with the hypothesis presented by Miles (*1*).

After a period of time in vitro, cultures 1 and 2 in the table consisted of reticular cells with a wide range of chromosome numbers. The modes significantly varied from the diploid number. Cultures 3 and 4 consisted of reticular cells and lymphoblastoid cells which were growing in suspension or involved in periopolesis and emperiopolesis. Wide ranges of chromosome numbers were also observed in these cultures, but modality approached the diploid number. Numbers 5 and 6 consisted of rapidly dividing lymphoblastoid cells in suspension cultures. These cultures had diploid modes and less deviation in the number of chromosomes per cell, although apparently polyploid, nondividing cells have been reported in these cultures (*16*).

Five of the cultures included in this report were initiated from tissue diagnosed as the nodular-sclerosing type of Hodgkin's disease. The sixth culture (#4) was initiated from tissue diagnosed as the mixed-cellularity type. It differed in that a lower percentage of dividing cells was present in the original sample, but rapidly dividing lymphocytes were present at an earlier time in vitro. The culture presently consists of lymphoblastoid cells growing in suspension, the reticular cells having disappeared after approximately 2½ months. The mode and range

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TABLE I.—*Chromosome enumerations of cell cultures derived from patients with Hodgkin's disease*

	Initial					Established				
Culture	Days	Range	Mean	Mode	Modal %	Months	Range	Mean	Mode	Modal %
1	3	36–47	43	46	24	3	34–180	37	53	12
2	3	23–73	44	46	38	3	17–85	40	38	16
3	3	27–46	36	46	16	4	17–88	44	46	10
4	3	35–51	44	46	32	2	28–114	43	45	12
5	3	19–92	45	46	48	3	19–46	35	46	14
6	2	29–80	51	46	18	4	22–54	42	46	24

of chromosome numbers are now similar to those observed in cultures 5 and 6.

Peckham and Cooper (*7*) performed photometric determinations of cells from lymph nodes. They found that abnormal reticulum cells are present with high DNA values and that some lymphoblastoid cells are aneuploid. The chromosome enumerations of the initial stages in culture are consistent with these findings, considering that reticulum cells were dividing in the cultures with wide ranges in chromosome numbers but not in those cultures in which the range is ± 2n/cell. The long-term cultures revealed similar distributions in relation to the types of cells present at the time of sampling.

Two interpretations may be given to the chromosomal complements of the cells continuing in vitro. One would like to suppose that the abnormal cells of the original tissue continued in culture, essentially preserving their variation in chromosome number. However, the growth characteristics of the cells may have selected lymphoblastoid cells with ± 2n chromosomes/cell while favoring the failure of cytokinesis and heteroploidy of the stationary reticulum cells. Both interpretations are consistent with the hypothesis of Miles (*1*), and either interpretation may be of value in assessing the cellular events in vitro.

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SUMMARY OF INFORMAL DISCUSSION ON CHROMOSOME STUDIES

H. Rappaport asked the speakers whether they ever used normal lymph nodes obtained from an abdominal laparotomy as controls. Had they compared lymph nodes which were involved and which were not involved by Hodgkin's disease with respect to chromosome abnormalities in short-term, 24-hour culture?

C. P. Miles replied that he had not done cultures. As far as controls are concerned, he had studied a number of hyperplastic lymph nodes as have a number of other people, and the general finding is that they do not show chromosome abnormalities—they show simply apparently normal, diploid cells. From the reports in the literature, people who have used cultures get the same results. Some have done both direct preparations and short-term cultures, and they get the same results on the cultures as in the direct preparations.

A. D. Mitchell concurred in this view.

Rappaport stated that in his own laboratory all normal controls were also negative, but only short-term cultures of <24 hours had been used. He and his co-workers observed one case where positive histologic findings were not discovered in the spleen until after a positive karyotype report was obtained. In this instance, karyotype analysis was helpful before routine pathologic examination.

R. F. Dorfman asked Doctor Rappaport whether the case that he had commented on was a case of mycosis fungoides, rather than a case of Hodgkin's disease.

Rappaport stated that it was a case of Hodgkin's disease with lymphocytic depletion involving cervical lymph nodes, and a staging operation was done. A similar situation did occur in a case of mycosis fungoides in which the karyotype from the skin biopsy showed an abnormality. The lymph node biopsy taken 3 months later had the same karyotype abnormality as the skin biopsy but was not histologically diagnostic of mycosis fungoides. A year and a half later, another lymph-node biopsy showed classical mycosis fungoides.

Invited Discussion: Cell Culture Studies of Hodgkin's Disease [1,2]

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IN VITRO studies of neoplastic and normal cells may indicate cellular interrelationships and processes occurring in vivo. The cell culture studies reported by Sanders (*1*) are welcomed, as relatively few tissue culture investigations have been performed with material from individuals with Hodgkin's disease (*2–5*). In the first reported observations, Grand (*6*) described lymphocytes, reticular cells, and giant cells as appearing in the early stages in cultures. Approximately 20 years later, other investigators described similar cells in culture. However, there is a lack of agreement concerning the terminology and identification of these cells and also concerning the cellular processes occurring in vitro.

Lymphocytes, including lymphoblastoid forms, have been described by the various investigators. They are included in the category of "round cells" described by Sanders and associates (*1*). The reticular cells (*3–6*) appearing in culture have also been termed "attached cells" (*1, 2*) and "fibroblastic cells" (*12*). Grand (*6*) observed 2 types of giant cells in culture. He described the first as a Reed-Sternberg-like cell. Others have termed similar cells "histiocytes" (*7*), "monocytes" (*8*), or "macrophages" (*4*). The second form of giant cell is termed a "multinucleated giant cell" by all who have observed them in culture.

In discussing the cellular processes occurring in vitro, I include an additional study of long-term cultures derived from tissues involved with Hodgkin's disease. These cultures were initiated from minced splenic tissue obtained from patients who underwent staging laparotomies at Stanford University Medical Center during 1971. Histologic diagnoses of the original tissues included: one example of the lymphocyte-depletion (LD) type; two of the mixed-cellularity (MC) type; 15 of the nodular-sclerosing (NS) type; and 6 spleens with no histologic abnormality. In addition, several cultures were initiated from various types of lymphomas, from lymphoma patients with no histologic abnormality of the spleen, and from one "normal" spleen obtained following trauma to a patient with no history of Hodgkin's disease or lyphoma.

All tissue cultures were maintained in Falcon flasks in McCoy's 5a medium (modified), supplemented with 20% fetal calf serum (GIBCO, Berkeley, Calif.). For the first 6 weeks of cell culture, the medium was replaced twice weekly by resuspending any unattached cells. After this time, routine subculturing was accomplished by combining the cells removed by trypsinization with the cells in suspension; the total population was then transferred to new culture flasks. Each culture was observed closely for approximately 6 months; phase-contrast photomicroscopy and fixed and stained duplicate cultures grown on coverslips were used. I presented chromosomal analyses of some of the cultures in a preceding paper (*9*); some of the electron microscopy findings were described by Dorfman et al. (*10*).

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[3] The Hodgkin's disease cell culture studies were performed in association with Dr. Ronald F. Dorfman at the Stanford University School of Medicine. The multinucleation experimentation was conducted under the direction of Dr. T. C. Hsu, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025.

Lymphocytes were observed at the time of initiation, and a viable portion of these cells remained for approximately 6 weeks, except in one culture derived from a spleen with the LD type of Hodgkin's disease. In approximately a third of the cultures, periopolesis and emperiopolesis (*11*) of the lymphocytes were observed during this time. In 6 of these cultures, the lymphocytes began to grow more rapidly and developed the ability to grow in suspension without the presence of a feeder layer of cells. This occurred as early as 3 weeks in the culture derived from a normal spleen and in one culture from a lesion of Hodgkin's disease, MC type. This phenomenon occurred by the 5–7th week in vitro for 4 cultures from tissue diagnosed as the NS type of Hodgkin's disease. An additional culture, obtained from NS tissue, has continued to produce viable lymphoblastic cells, which, however, cannot be propagated without a feeder layer. Binucleated cells were occasionally observed in the suspension cultures.

The observations of these cultures are consistent with the interpretation of an "ecologic" adaption of the lymphocytes to a rapid growth rate and the ability to grow in suspension (*7*) rather than a "spontaneous transformation" from other types of cells (*2, 5, 12*). Therefore, one wonders if observations by Dr. Sanders of lymphocytic cells emerging from otherwise enucleate cytoplasm may be related to lymphocyte cytotoxicity or to observations that lymphocytes may attach to fragments of cytoplasm and cellular debris (*13*).

Reticular cells grew out from the minced tissue after 2–7 days in vitro. The reticular cells persisted in all cultures, except the 6 in which the percentage of these cells was gradually reduced in relation to the more rapidly dividing lymphocytes. A high proportion of binucleated and multinucleated forms were observed in the early cultures and have continued to be observed over a period of time. The proportion of reticular cells with multiple nuclei also increased when the cultures were allowed to become overgrown before subculturing or were subcultured with resulting high concentrations. I observed the same occurrence in normal fibroblasts and demonstrated that the percentage of multinucleated cells is directly related to cell concentration (*14*). This form of multinucleation was shown to be a result of c-mitosis (*15*) followed by a failure of cytokinesis.

Giant cells also appeared in the early stages in vitro. Some could be transferred with the cells in suspension during the 1st week in culture. Usually they spread over the surface of the culture flask to assume a rounded shape with a prominent and deeply staining cytocentrum and an eccentric nucleus. A continuum of cell sizes and number of nuclei per cell was observed from the smaller, mononuclear forms to large, multinucleated giant cells. These cells were in highest proportion in cultures obtained from the normal spleen, from tissue diagnosed as LD and MC types, and from the cellular phase of NS Hodgkin's disease. They persisted in culture for no longer than 6 weeks, when they were overgrown by the reticular cells and did not survive the use of trypsin.

The multinucleated giant cells are morphologically similar to "hyperlobated lacunar cells" seen in the NS type of Hodgkin's disease. The many nuclei of the giant cells were also similar in appearance and staining characteristics to lymphoblastoid cells. It has been suggested that they may arise from a faliure of cytokinesis (*6*) or by the fusion of smaller cells (*7, 8*). I would like to suggest a third hypothesis: That the multiple nuclei are those of lymphocytic cells engaged in emperiopolesis.

Autoradiographic studies using tritiated thymidine revealed little DNA synthesis within the first 2 days in culture. Then most labeled cells were lymphocytic with some labeling of the reticular cells. The only uptake of tritiated thymidine by the third group of cells was in the multiple nuclei of the giant cells in which the degree of labeling was equivalent to that of lymphocytes in the same samples.

The reports of cell cultures from Hodgkin's disease material have listed various types of cells as predominating and continuing in culture. This may be ascribed, in part, to the techniques used to initiate and continue the cells in culture and, perhaps, to the methods of observation. However, none of these cell types is exclusive to Hodgkin's cultures. Similar cells and patterns of growth have been observed in cultures derived from various sources (*7*), including those from normal lymphatic tissue.

One therefore returns to the original purpose of the tissue culture investigations—to further the understanding of cellular processes and interrelationships in Hodgkin's disease. As the cellular elements are more precisely characterized and identified, it should be possible to reach more complete agreement between the areas of histology and tissue culture, concerning those elements appearing in vitro corresponding to those observed in the tissue of origin. Further tissue culture studies on the processes of periopolesis, emperiopolesis, and the formation of multinucleate cells will then be of value in understanding the importance and implications of such processes when occurring in vivo.

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Invited Discussion: In Vitro Study of Multinucleated Cells in Hodgkin's Disease [1,2]

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PREVIOUSLY PUBLISHED accounts of DNA synthesis (*1*) and mitotic activity (*2*) in cells comprising Hodgkin's tissue lesions indicate that the Reed-Sternberg cell is a nonproliferative or end-stage cell. Furthermore, recognition of cells morphologically identical to the Reed-Sternberg cell in infectious mononucleosis (*3*), a benign disorder, raises further doubts as to the proliferative potential and origin of these distinctive cells. Lukes (*4*) and others (*5, 6*) recently emphasized the significance of certain multinucleated cells thought to be variants of the Reed-Sternberg cell associated with specific histologic types of Hodgkin's disease; the nature of these cells is similarly unknown.

The present study attempts to elucidate the derivation and proliferative properties of multinucleated cells in long-term cell cultures derived from Hodgkin's tumor nodules by use of the techniques of thymidine labeling and mitotic arrest. A detailed quantitative analysis of the data is presented elsewhere (7).

MATERIALS AND METHODS

Explants from splenic tumor nodules of untreated patients with nodular-sclerosing (NS) (2) and mixed-cellularity (MC) (2) types of Hodgkin's disease were maintained for 4–6 weeks, at which time suspension cultures were established in a manner similar to that described for other human lymphoid cell lines (*8–10*). Stationary suspension cultures were grown in Leibovitz's L-15 media containing 15% fetal calf serum in a 5% CO_2 atmosphere at 37° C. Cultures were divided and fresh media renewed twice weekly, maintaining the population density at 5×10^5 to 2×10^6 cells/ml.

After 3–7 months in culture, cells were inoculated at densities varying from 5×10^5 to 10^6/ml and incubated either in media containing 1 μCi/ml tritiated thymidine or 1 μg/ml vinblastine. After a 1-hour incubation with tritiated thymidine, cells were rinsed in Hanks' solution and either smeared onto clean glass slides, which were then air-dried and placed into absolute methanol, or fixed for 30 minutes in cold, phosphate-buffered 3.6% glutaraldehyde, dehydrated, and embedded in glycol methacrylate. Then 1.5–2.0μ methacrylate sections were dipped into Kodak NTB2 liquid photographic emulsion at 40° C in total darkness, exposed for 4–5 days at −70° C in light-tight boxes, and developed in a single run. The preparations were processed in Kodak D-19 developer for 3 minutes and stained with 0.05% toluidine blue. The fixed and dried smears were processed in a similar manner, but were exposed for only 1 day and stained with either toluidine blue or a Romanovsky stain. A nucleus was counted as labeled only if it was covered by $\geqq 5$ silver grains (background counts <0.5 grains/100μ^2).

Cells incubated with vinblastine were harvested at 0 time and 8-hour intervals thereafter for light and electron microscopy.

Enzyme cytochemistry was performed on un-

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stained air-dried smears of freshly harvested cells. Acid and alkaline phosphatase activities were determined with methods of Burstone (*11, 12*). Esterase activity was measured with both α-naphthyl acetate (*13*) and naphthol AS-D chloroacetate (Sigma Chemical Co., St. Louis, Mo.) (*14*) as substrates. The presence of myeloperoxidase was also assessed (*15*).

RESULTS

Months of observation of cell cultures derived from splenic tumor nodules of untreated patients with the MC and NS types of Hodgkin's disease demonstrated the persistent appearance of both mononucleated and multinucleated cells having a diverse lymphoid morphology. Similar observations have been made by Moore and associates (*16, 17*) in describing numerous human hematopoietic cell lines derived primarily from the peripheral blood of patients with a variety of malignant and benign diseases as well as from normal individuals. In the present cultures, however, there also persisted atypical mononucleated and multinucleated cells with large, prominent nucleoli which were sometimes morphologically indistinguishable from the Hodgkin's or Reed-Sternberg cells (figs. 1, 2). The ultrastructure of these cells conformed to that originally described by Bessis (*18*). Other multinucleate cells with more numerous nuclei and less conspicuous nucleoli appeared to correspond to variants of the Reed-Sternberg cell described by Lukes (*4*) and others (*5, 6*) (fig. 3). Occasionally adjacent nuclear lobes were connected by narrow segments of nuclear material (fig. 1).

Multinucleate cells comprised an average of 3% of the total cell population. They labeled with tritiated thymidine, though not as often as their mononuclear counterparts. In most instances the grains were distributed uniformly over the various nuclei (figs. 2, 3). Uncommon examples of asynchronous nuclear labeling were detected, however. In the latter, the majority of nuclei was evenly labeled, whereas a minority (usually only one) was unlabeled.

Mitoses were detected in 1–3% of the cells. When vinblastine was added to cultures, most cells were arrested in mitosis after 24 hours. Abnormal multipolar mitoses were present in some mononuclear cells in untreated cultures (fig. 4). Multiple discrete and synchronous mitoses were observed within some multinucleated cells (figs. 5, 6). However, occasionally it was not possible to distinguish between mitoses occurring in multinucleate cells and apparently polyploid mononucleate ones (fig. 7). Therefore, the frequency of mitoses in multinucleate cells could not be accurately assessed. On no occasion could one demonstrate a multinucleated cell in which one or more nuclei were in mitosis while the remainder were in interphase; this was true, even in cultures exposed to vinblastine for up to 24 hours. Similarly, all multinucleate mitotic cells failed to show evidence of cytokinesis (cytoplasmic division leading to cellular multiplication).

Acid phosphatase activity was present in small amounts primarily in the Golgi region of both mononucleated and multinucleated cells. This corresponded to the presence of alveolate vesicles in these foci. By contrast, alkaline phosphatase, myeloperoxidase, and nonspecific and chloroacetate esterases were not detected in these mononucleated and multinucleated cells.

DISCUSSION

The continued presence of multinucleated cells in cultures derived from Hodgkin's tumor nodules initiated up to 7 months previously requires that they originated either from multinucleated cells in the initial explants through repeated cell divisions or de novo from the mononucleated cells or possibly in both ways.

Were the multinucleated cells to result from fusion of multiple mononucleated forms, one would expect to find some cells in which nuclei of different morphologic types were coexistent; this never occurred. Furthermore, nuclear asynchrony would probably be a frequent event (*19, 20*), whereas in these cultures it was exceptional. Lastly, in electron micrographs incomplete cell membranes were never observed between adjacent nuclei of multinucleated cells.

Alternatively, there was evidence that the multinucleated forms developed as a result of abnormal multipolar mitoses and repeated nuclear division in the absence of subsequent cytoplasmic division (cytokinesis). Contrary to previous reports, the present study indicates that the multinucleated cells were capable not only of DNA synthesis but also of subsequent nuclear division.

Preliminary cytogenetic studies revealed some polyploidy and aneuploidy in each of 2 cell lines sampled, though each had a mode of 46 chromosomes (*21*). Aneuploidy, most commonly expressed as group C monosomy, occurred in polyploid as well as peridiploid cells. Endoreduplication was also frequently observed (fig. 8). Future studies will include karyotyping of initial explants.

Previous histochemical studies by Dorfman (*22, 23*) suggested that the Reed-Sternberg cell is more closely related to the lymphocyte than the histiocyte. The observations reported here support this concept. Lymphocyte and Hodgkin's cell types continued to coexist and appeared to be interrelated through transitional forms. Similarly, they could not be differentiated on the basis of their cytochemical properties. Multinucleated cells, including the atypical Reed-Sternberg-like forms, possessed cytochemical properties distinguishing them from normal and neoplastic histiocytes and granulocytes (*13–15, 22, 23*). They lacked the nonspecific esterase activity of histiocytes and the chloroacetate esterase and peroxidase activities of granulocytes (*13*).

Cultures of normal spleen have been initiated, but these have not been observed for a long enough time to permit comparison with the long-term Hodgkin's cultures presented here. Nevertheless, a recent report (*24*) on the development and behavior of multinucleated cells arising in chick spleen cultures indicated that these cells were derived from the fusion of macrophages or undifferentiated reticular cells. Atypical nuclear features in these multinucleated cells were not illustrated or described.

The present study indicates that the multinucleated cells in Hodgkin's disease continue to synthesize DNA and accomplish nuclear division under conditions of continuous tissue culture. However, cytokinesis of these cells was not observed. Whether the presence of the multinucleated cells is due only to the failure of cytokinesis, accompanied by continued nuclear division, or whether it is due also to whole cell multiplication remains to be determined. Further assessment of the proliferative properties of these cells with use of cloning techniques may resolve this question.

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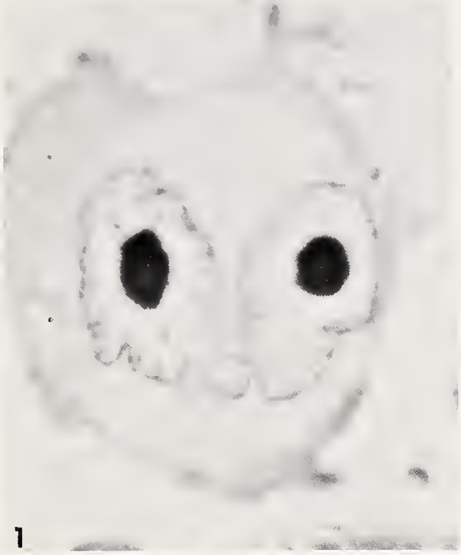


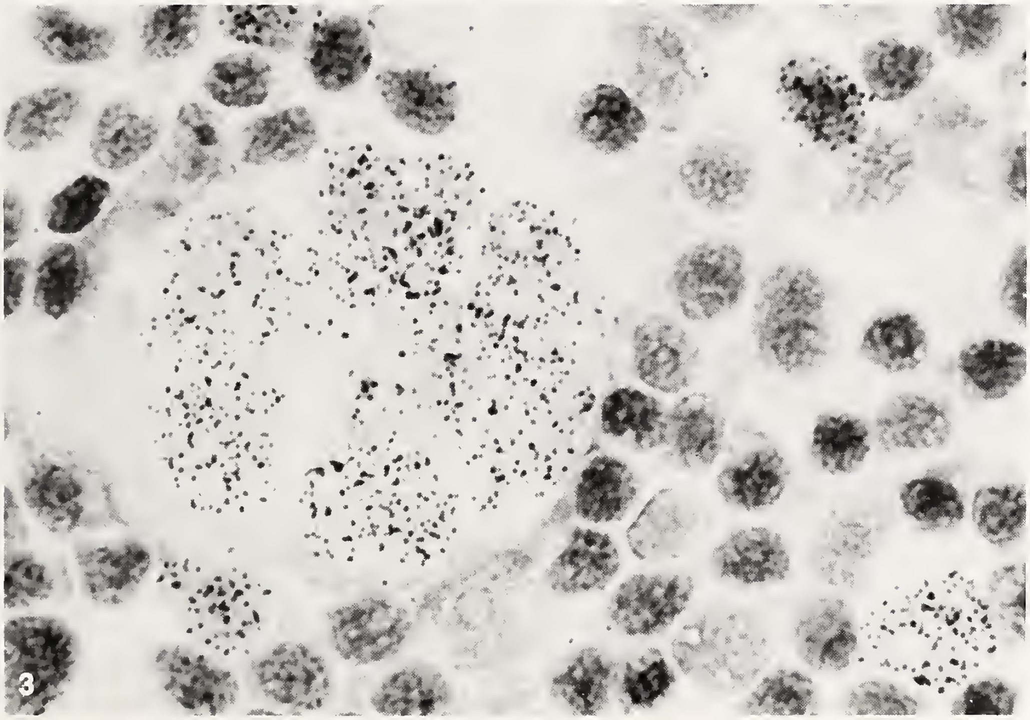
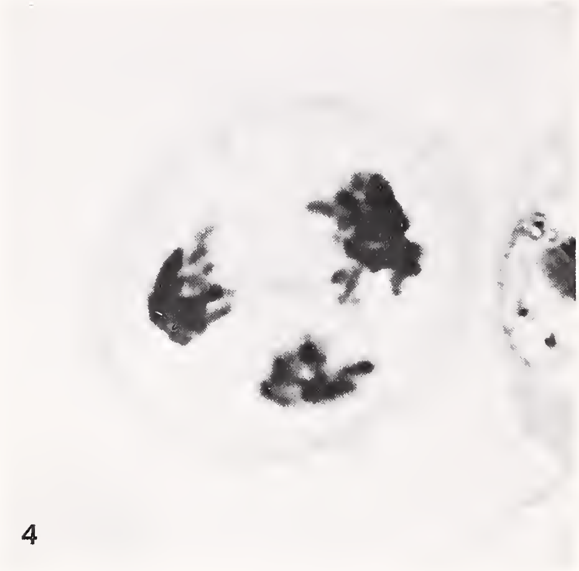


FIGURE 1.—Atypical cell with a mirror-image nucleus and prominent inclusion-like nucleoli—from suspension cell culture initiated 6 months earlier; spleen, Hodgkin's disease, NS type. Glycol methacrylate section (2 μ). Toluidine blue. × 900

FIGURE 2.—Binucleated cell from a 6-month-old culture labeled by a 1-hour pulse with tritiated thymidine (1 μCi/ml). Silver grains (in focus) are above focal plane of cell. Glycol methacrylate section (μ). Toluidine blue. × 900

FIGURE 3.—Multinucleated cell and several mononucleated cells are labeled with thymidine in smear prepared from 7-month-old suspension cell culture of Hodgkin's disease, NS type. Multinucleated cell resembles one of the Reed-Sternberg cell variants associated with this histologic type. Toluidine blue. × 900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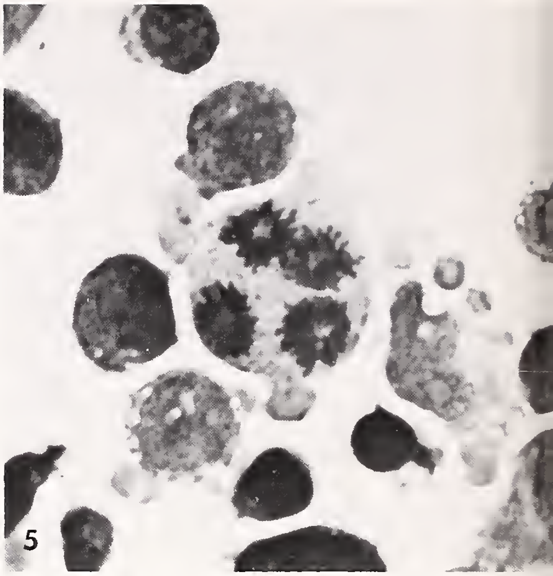
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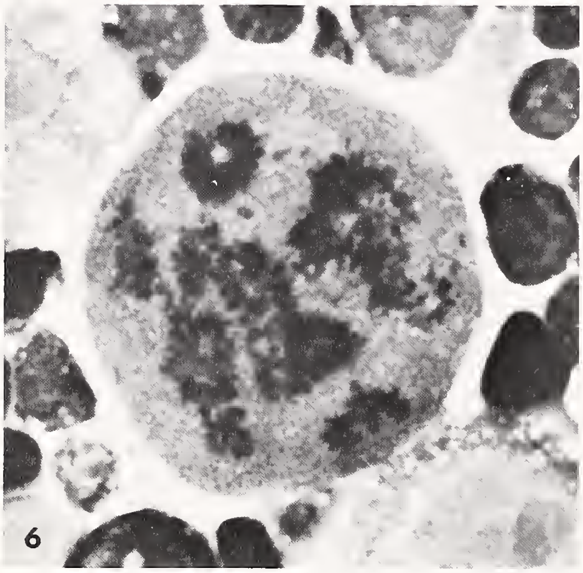
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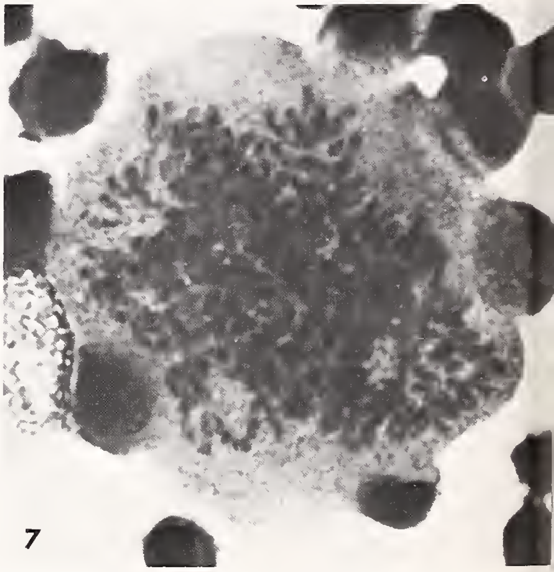
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Figure. 4.—Abnormal multipolar mitosis in mononucleated cell from Hodgkin's cell culture. Glycol methacrylate section (2 μ). Toluidine blue. × 2000

Figure 5.—Example of synchronous mitoses in a small multinucleated cell from 7-month-old Hodgkin's cell culture. Smear preparation. Wright's-Giemsa. × 900

Figure 6.—Multiple simultaneous mitoses in a large multinucleated cell; Hodgkin's splenic tumor nodule, 6-month-old suspension culture. Smear preparation. Wright's-Giemsa. × 900

Figure 7.—It is not clear whether this polyploid mitotic cell was mono-nucleated or multinucleated; Hodgkin's disease, MC type, 4-month-old suspension culture. Smear preparation. Wright's-Giemsa. × 900

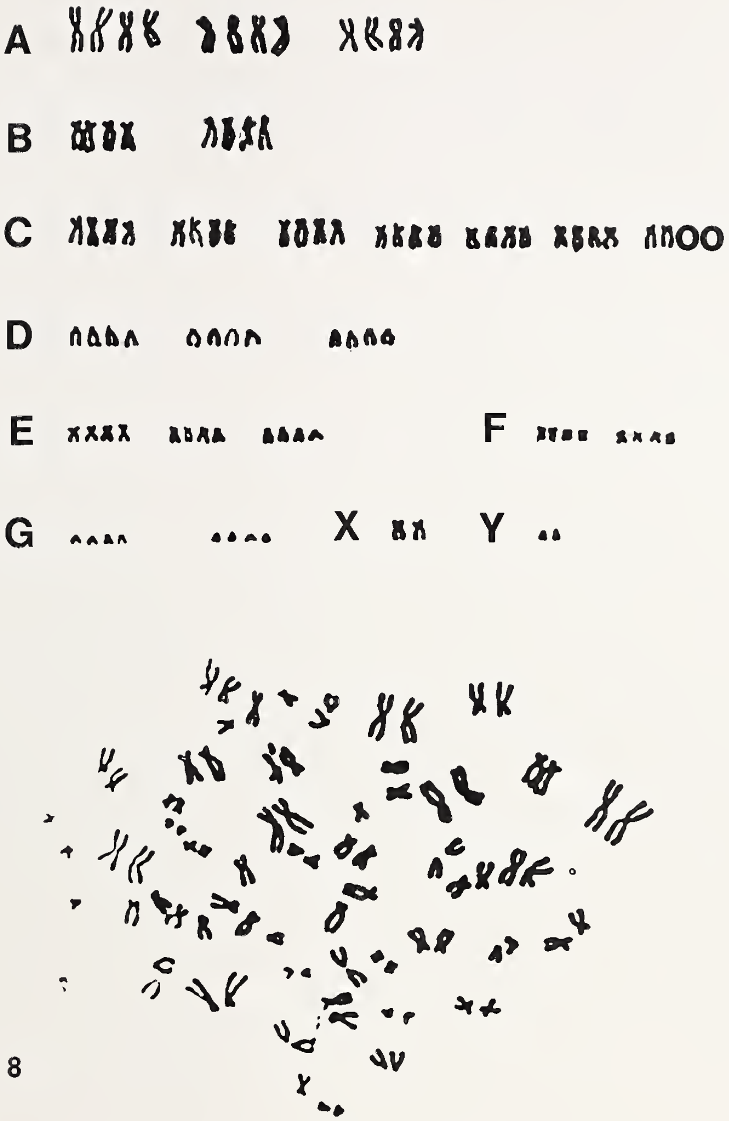

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Figure 8.—Metaphase spread (*bottom*) and karyogram prepared from a 5-month-old Hodgkin's culture illustrate endoreduplication, the phenomenon whereby polyploidy results from chromosomal replication in the absence of nuclear division. Forty-five diplochromosomes (paired chromosomes, best seen at *upper right* of metaphase spread) are present. Karyogram reveals a deficiency of one of these chromosome pairs (a replicated autosome), probably from the C group, as indicated by the O's at the *far right* of group C.

SUMMARY OF INFORMAL DISCUSSION ON CELL CULTURE STUDIES

K. E. Hellström spoke on behalf of his wife, Dr. I. Hellström, who was unable to attend the meeting. They had been studying human tumor immunology during the last 4 years in Seattle. When they started this work, one of their greatest problems was to know whether the cells they grew in vitro from the tissues of cancer patients were really the tumor cells that they were interested in. The criterion they used was growth in a 3-dimensional pattern rather than just as a monolayer. They hoped that these were tumor cells, and they started to test whether these cells were killed by lymphocytes from patients with cancer.

The Hellströms had worked out the model system in animals previously and knew in the animal situation that tumor cells could be cultivated under these conditions, since they could transplant the cultivated cells back to animals and observe their growth in vivo into tumors that killed these animals.

They found in the human situation that the patient's lymphocytes killed cultivated autochthonous tumor cells, and they also found to their great surprise that the lymphocytes also killed tumor cells from other patients with the same types of tumors. Thus, evidence was obtained for the existence of cross-reacting antigens among histologically similar tumors. For example, colon carcinomas cross-reacted with each other; breast carcinomas cross-reacted with each other; melanomas cross-reacted with each other. However, different tumor types did not cross-react.

Recent studies performed by the Hellströms with cultured cells from lymph nodes and spleens of patients with Hodgkin's disease have shown that lymphocytes from some patients with Hodgkin's disease which was no longer clinically active were cytotoxic to target cells from Hodgkin's tissue, but not to normal cells or to other types of tumors. Most patients with active Hodgkin's disease lacked such detectable lymphocyte reactivity.

Ultrastructural Studies of Hodgkin's Disease [1,2]

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SUMMARY—A comprehensive ultrastructural study was undertaken of lesions derived from the spleen and lymph nodes of patients with Hodgkin's disease undergoing surgical staging procedures at Stanford University Medical Center. Recent concepts concerning the nature of the Reed-Sternberg cell were explored. Studies of surgical material and of cells established in tissue culture showed the presence of blastlike cells resembling so-called immunoblasts (transformed lymphocytes). Furthermore, ultrastructural characteristics of mononuclear and hyperlobated Reed-Sternberg cells provided some evidence supporting the concept that these cells may be derived from the transformed lymphocyte. Concentrations of lymphocytes and in some instances eosinophils, around Reed-Sternberg cells, and the observation of uropod formation and adhesion between the cytoplasm of lymphocytes and "Hodgkin's cells," suggest that interactions between these cell types are important in the pathogenesis of Hodgkin's disease.—Natl Cancer Inst Monogr 36: 221–238, 1973.

REVIEW OF the literature on the electron microscopy of Hodgkin's disease reveals a paucity of information on this subject. The earliest descriptions of the ultrastructure of the Reed-Sternberg cell appear in reports by André et al. in 1955 (*1*), Frajola et al. in 1958 (*2*), and Braunsteiner et al. in 1957 (*3*). More comprehensive descriptions appear in the monograph by Bernhard and Leplus (*4*). There have been few studies relating ultrastructure to the morphologic descriptions of Lukes and Butler (*5*). Mori and Lennert (*6*) included brief descriptions of cells observed in the mixed-cellularity (MC) and nodular-sclerosing (NS) types of Hodgkin's disease. More recently Archibald and Frenster (*7*), at this institution, examined material from several cases of the NS type and have concentrated on specific aspects, in particular, interrelationships of cell types in Hodgkin's disease.

We have undertaken a comprehensive study to correlate the ultrastructure of Hodgkin's disease with the histologic types described by Lukes and Butler (*5*). This paper will not attempt to encompass an overall description of the ultrastructure of this disease, but will concentrate specifically on a preliminary study of the Reed-Sternberg cell and its possible relationship to other cell types observed in the stroma of the lesions examined.

We have also initiated tissue cultures of cells from these lesions and will present preliminary observations on the ultrastructure of cells established in culture for varying periods.

The Reed-Sternberg cell has frequently been referred to as an atypical reticulum cell or neoplastic histiocyte (*8*). Recent reports describing the observation of Reed-Sternberg-like cells in diseases other than Hodgkin's disease (*9–11*), and particularly in non-neoplastic diseases such as

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infectious mononucleosis, have raised doubts about the specificity of this cell. They have also introduced new concepts regarding the nature of the Reed-Sternberg cell and its possible relationship to the transformed lymphocyte (*9, 12*). We are in the process of exploring the validity of these concepts. Our observations suggest that this intriguing hypothesis relating the Reed-Sternberg to the lymphocyte warrants further investigation.

MATERIALS AND METHODS

Lesions from 46 spleens and 10 lymph node biopsies derived from patients with Hodgkin's disease undergoing surgical staging procedures at the Stanford University Medical Center were examined in the electron microscope. Of these lesions, 38 were of the NS type, 7 of the MC type, and 2 of the lymphocyte-depletion type. As controls we also examined uninvolved spleens and lymph nodes removed from patients with diagnostic evidence of Hodgkin's disease elsewhere, together with apparently normal spleens removed following traumatic rupture or during surgery for other abdominal disease. Surgical material for electron microscopy was fixed in buffered paraformaldehyde-glutaraldehyde fixative (*13*), postfixed in osmium tetroxide, stained with uranyl acetate, and embedded in Epon after dehydration in graded alcohols. Thin sections cut on an LKB ultratome were placed on parlodion-coated copper grids, stained with lead citrate, and examined in an HU-11a Hitachi electron microscope.

Methods for tissue cultures are described elsewhere (*14*).

For electron microscopy, centrifuged pellets were fixed in buffered glutaraldehyde for 30 minutes to 1 hour at 4° C. After being washed in cacodylate buffer, pellets were postfixed in osmium tetroxide stained with uranyl acetate and dehydrated in graded alcohols, followed by 6 changes of graded 2-hydroxylpropyl methacrylate, and embedded in Epon.

RESULTS

"Hodgkin's Cells"

In the light microscope, the mononuclear and lobated cells of the Reed-Sternberg type are characterized by the presence of extremely large, inclusion-like nucleoli which appear uniformly acidophilic in sections stained with hematoxylin and eosin (fig. 1A). Electron micrographs showed large electron-dense and often bizarre nucleoli (figs. 2–4). In some nuclei these nucleoli were well circumscribed. Others had a filamentous branching configuration. The nucleoli were often localized to the central part of the nucleus, but also encroached upon the nuclear membrane for a considerable distance. The nucleoplasm of most cells was finely granular, and the chromatin was evenly dispersed throughout the nucleoplasm. However, occasional mononuclear and lobated cells showed a concentration of chromatin at the periphery in relation to the nuclear membrane. This was particularly evident in cells undergoing degenerative changes (fig. 2). Although apparent binucleation was observed, many cells, thought to be binucleated or multinucleated in the light microscope, were seen in the electron microscope to have a single, hyperlobated nucleus with multiple nucleoli. This was also evident in thick Epon sections stained with toluidine blue (figs. 1B, 1C). While the nuclei of both mononuclear and hyperlobated cells were frequently uniformily round, many cells had complex convoluted and indented nuclear membranes (figs. 3–5).

The cytoplasm of both mononuclear and hyperlobated Reed-Sternberg cells varied. In some instances, in all 3 histologic types examined, there was a distinct paucity of organelles (fig. 3). The cytoplasm contained numerous free ribosomes and often polysomes with little rough endoplasmic reticulum. Mitochondria varied in number, being few in some cells (fig. 3) but quite numerous in others (fig. 4). In the latter cells, they were often dilated and the cristae were fragmented. The Golgi apparatus similarly varied, being poorly developed in some cells and prominent in others. Although occasional small dense bodies were noted, no distinct lysosomal structures and no phagosomes were observed in characteristic mononuclear or hyperlobated Reed-Sternberg cells. Nuclear blebs enclosing cytoplasmic material were observed in both mononuclear and hyperlobated cells (figs. 2, 5, 6).

Most cases from which we obtained material were of the NS type. In the light microscope there is considerable variation in the morphology of Reed-Sternberg cells and so-called "hyperlobated lacunar cells," which Lukes and Butler considered to be variants of the Reed-Sternberg cell. We were similarly impressed by the variability of cells of this nature observed in the electron microscope. Cells which we interpreted as "Hodgkin's cells" appeared to be of two main types. One type had a thin rim of cytoplasm with a distinct paucity of organelles and of rough endoplasmic reticulum, but with numerous free ribosomes and often polyribosomes (figs. 3, 5). These cells had small rod-shaped mitochondria frequently polarized in the region of the Golgi apparatus, which was poorly developed. The nuclei, while possessing large electron-dense nucleoli, sometimes filamentous in shape, usually had finely dispersed chromatin. However, others showed some condensation of chromatin around the nuclear membrane. The second cell type possessed similar nuclear features but the cytoplasm differed markedly. The cytoplasm was abundant and contained many organelles including both smooth and rough endoplasmic reticulum, a prominent Golgi apparatus, and many mitochondria, randomly distributed throughout the cytoplasm (fig. 4). Mitochondria were often dilated and contained fragmented cristae. Cytoplasmic fibrils were also noted. Neither cell type contained lysosomal structures.

Cells, which we interpreted as histiocytic by virtue of the abundance of cytoplasm and associated organelles, including lysosomes and phagosomes enclosing phagocytosed material, often contained many free ribosomes and polysomes. The nuclei of such cells frequently possessed large electron-dense nucleoli. Both varieties of Reed-Sternberg cells and the above-described histiocytes were seen in all 3 types of Hodgkin's disease examined.

Lacunar Cells

Lukes and Butler described the presence of cells with hyperlobated nuclei and somewhat inconspicuous nucleoli which, in formalin-fixed tissue, appeared to lie within "lacuna-like spaces." This appearance results from retraction of the cytoplasm of these cells and is an artifact observed only in formalin-fixed tissue but not in Zenker-fixed material. Nevertheless the morphologic appearance of these cells in the light microscope is distinctive and is characteristic of NS Hodgkin's disease. Lukes and Butler considered these cells to be variants of the Reed-Sternberg cell. Ultrastructurally, the nuclei of these cells were similarly hyperlobated with complex convoluted and indented nuclear membranes (fig. 5). Nucleoli were frequently less prominent than those observed in characteristic Reed-Sternberg cells, though they often had a filamentous pattern. The cytoplasm of hyperlobated cells varied (as described above).

Lymphocytes

These cells were often clustered around both mononuclear Hodgkin's cells and Reed-Sternberg cells, frequently associated with eosinophils and occasional plasma cells (figs. 6–8). The cytoplasmic membranes of lymphocytes were frequently in close apposition with those of the Hodgkin's cells. Projections of the cytoplasm resembling so-called "uropods" were often prominent. Furthermore, there was frequently a complex, convoluted, and interdigitating pattern of the cytoplasmic membrane of lymphocytes, histiocytes, eosinophils, and Reed-Sternberg cells (fig. 6).

The ultrastructure of most lymphocytes conformed to the description of normal lymphocytes by virtue of the condensation of chromatin around the nuclear membrane, a well-defined electron-dense nucleolus, often with a central zone of moderate density and a thin rim of cytoplasm, poor in organelles (fig. 7). Mitochondria were characteristically polarized in most cells and frequently located in association with the Golgi apparatus and centrioles. Indentations in the nuclear membrane were often noted in this region of the cell. Of particular interest to us was our observation of tubular arrays within dilated ergastoplasmic sacs, invariably in the region of the Golgi apparatus and often adjacent to mitochondria (fig. 9). These arrays were bounded by membranes in continuity with the endoplasmic reticulum. Their diameters were approximately 220 A. At the time of writing we have observed these structures only in lymphocytes in both biopsy material and in cells established in tissue culture, as will be described below.

Close to and often adjacent to normal lymphocytes, distinctive mononuclear cells were frequently observed. They were ultrastructurally different from the above-described "Hodgkin's cells" and showed the following morphologic features (figs. 7, 8). Their nuclei were larger than those of adjacent lymphocytes, and the nuclear membranes were often convoluted with identations of cytoplasmic material: Chromatin was more finely dispersed throughout the nucleoplasm, though some nuclei showed condensation of chromatin along the nuclear membrane similar to that observed in adjacent lymphocytes. Nucleoli, single or multiple, varied in size and shape, some being well circumscribed and electron-dense and others having a filamentous or cribriform configuration. These cells possessed variable amounts of cytoplasm containing many free ribosomes and often polysomes, some smooth and occasionally rough endoplasmic reticulum, and small mitochondria. Some mitochondria were tubular or rod-shaped, and others were rounded and somewhat dilated, with ruptured cristae. The possible relationship of such cells to adjacent lymphocytes is discussed below.

Ultrastructural Observations in Cells Established in Tissue Culture

Although these studies are preliminary, we believe it pertinent to present our observations in relation to those made on biopsy material. These remarks concern blastlike cells similar to those described by Eisinger et al. (*15*) and observed by us within the first few weeks of tissue culture, together with cells established in culture for as long as 5½ months. While some of the latter show morphologic features closely resembling those of the Reed-Sternberg cell (fig. 10), we are fully aware that extrapolation of morphology of cells in tissue culture with that observed in material prepared for light microscopy or electron microscopy may be fallacious.

Pellet preparations of splenic lesions maintained in culture for 5½ months contained a variety of cell types. Prominent among these were blastlike cells which resembled transformed lymphocytes (figs. 11, 12). The cytoplasm of these cells was thin and rich in free ribosomes and contained varying amounts of smooth and rough endoplasmic reticulum with occasional small dense bodies. The nuclei of some showed condensation of chromatin at the periphery, and nucleoli were prominent. Other cells had a more dispersed nucleochromatin pattern, and nuclear indentations were seen in the region of the Golgi apparatus. These cells also showed polarization of mitochondria. Furthermore, tubular arrays identical to those observed within lymphocytes described above were seen within ergastoplasmic sacs in the region of the Golgi apparatus (fig. 12).

Larger apparently binucleated cells with convoluted nuclear membranes, a fine chromatin pattern, and large electron-dense nucleoli within each nucleus were also noted (fig. 10).

We have not observed viral particles in either tissue culture material or in surgical material.

DISCUSSION

The nature of the Reed-Sternberg cell has been the subject of debate ever since its description by Sternberg (*16*) and Reed (*17*). (Other authors are alleged to have described this cell first, but none as satisfactorily as Sternberg and Reed; thus the eponym is justified.)

On morphologic grounds the Reed-Sternberg cell has been considered by many to be a reticulum cell or neoplastic histiocyte (*8*). Braunstein et al. (*18*) favored the latter concept on the basis of their enzyme histochemical studies. They reported strong activity of both acid phosphatase and nonspecific esterase in normal, reactive, and neoplastic histiocytes. They stated that, in general, the enzymatic activity of cells of the various histiocytic types of malignant lymphoma corresponded to that of their normal counterpart. They claimed also that large anaplastic cells and Sternberg-Reed cells of Hodgkin's disease also manifested a similar pattern histochemically. They implied, therefore, that the Reed-Sternberg cell was a bizarre neoplastic histiocyte. Utilizing similar histochemical methods, one of us (RFD) could not demonstrate significant activity of either nonspecific esterase or acid phosphatase in Reed-Sternberg cells (*19, 20*). Thus the conclusion reached was that Reed-Sternberg cells were probably derived from cells other than histiocytes. It was also suggested that the enzyme activity of Reed-Sternberg cells more closely resembled that of the lymphocyte than of the histiocyte.

Lukes and associates (*9*), Strum et al. (*11*), and others (*10*) recently described the presence of "Reed-Sternberg cells" in diseases other than Hodgkin's disease including non-neoplastic diseases. Of particular interest was the observation of Reed-Sternberg-like cells in infectious mononucleosis. Lukes et al. thus questioned the specificity of the Reed-Sternberg cell and suggested that it may not be pathognomonic of Hodgkin's disease. Lukes and Butler (*5*) emphasized that the diagnosis of Hodgkin's disease depends not only on the identification of Reed-Sternberg cells but also on the presence of an appropriate stromal component, which they have described in detail. The lymphoproliferative process in infectious mononucleosis is characterized by the presence of numerous so-called "immunoblasts" (transformed lymphocytes). Lukes and his associates (*12*) suggested that the evolution of the Reed-Sternberg-like cells in this disease can be traced from an innocuous-appearing small "immunoblast" through stages of various sizes and configurations to the large polyploid hyperlobated form. Thus they consider the Reed-Sternberg-like cell to be an altered "immunoblast." By inference they suggest that the Reed-Sternberg cell of Hodgkin's disease is also of immunoblastic origin. Peckham and Cooper (*21*) reported the presence of large basophilic transformed lymphocytes in the stroma of Hodgkin's disease and demonstrated evidence of cell division in this type of cell by virtue of their tritiated thymidine-labeling index. They demonstrated aneuploidy in cells which they termed "the Hodgkin's reticulum cell line," though most transformed lymphocytes appeared to fall within the normal diploid range.

What ultrastructural evidence is there that the Reed-Sternberg cell is derived from a lymphocyte, via the transformed lymphocyte or immunoblast? In their study on the fine structural alterations of interphase nuclei of antigenically stimulated lymphocytes *in vitro*, Tokuyasu et al. (*22*) described the following changes in these cells: "There is a gradual dispersion of the characteristic condensed nuclear chromatin. The nucleus enlarges, becomes somewhat lobulated, and the nuclear chromatin is finely dispersed throughout the nucleus. Furthermore 2–4 nucleolar profiles were not exceptional at 48 and 72 hours in culture." Elves et al. (*23*) also described in some detail the characteristic cytoplasmic features of the transformed lymphocyte, under the influence of phytohemagglutinin. The cytoplasm of blast cells at 72 hours contained much cytoplasm rich in ribosomes and mitochondria and showed endoplasmic reticulum in the form of small vesicles or dilated sacs. They also noted large electron-dense bodies which were membrane bound. The Golgi apparatus was well developed and in some cells quite extensive.

In exploring the possible validity of the concepts expounded by Lukes et al., we were impressed by the fact that the nuclei of many mononuclear and hyperlobated "Hodgkin's cells" resembled those of transformed lymphocytes. This applied equally to cells established in tissue culture. The variation in cytoplasmic ultrastructure might well be related to the degree of differentiation of these cells or might support a dual origin for "Hodgkin's cells" from both lymphocytes and histiocytes.

The blastlike cells we observed in suspension cultures are morphologically similar to cells that we observed in histologic sections within the stroma surrounding Reed-Sternberg cells in all types of Hodgkin's disease. These were described by Peckham and Cooper (*21*) as basophilic stem cells or transformed lymphocytes.

After identifying similar cells in tissue culture, Eisinger et al. (*15*) interpreted this observation as a phenomenon of "blastoid transformation" and suggested that this was associated with evidence of the emergence of a herpes-like virus. While we cannot comment on the validity of the latter observation, we question their interpretation that so-called blastoid transformation is a phenomenon occurring only in tissue culture, since we and others (*21*) observed identical cells in the stroma of Hodgkin's disease in imprint preparations and in histologic sections. These cells are strongly pyroninophilic and morphologically identical with immunoblasts seen in other antigenically stimulated lymphoid tissues.

We have observed polyploid cells after 5½ months in tissue culture, with large electron-dense nuclei resembling Reed-Sternberg cells. We are nevertheless fully aware of the dangers of interpreting these cells as such and make no claims to having established Reed-Sternberg cells in tissue culture. We appreciate that neither our ultrastructural findings nor our observations on the mode of growth of cells in culture have provided conclusive evidence of the nature or origin of the Reed-Sternberg cell. We are, however, utilizing other methods including kinetic studies, histochemistry, and cloning techniques to further investigate these concepts.

The significance and nature of the so-called tubular arrays or undulating tubules have also been the subject of controversy. Grausz et al. (*24*) described these structures in the glomerular endothelium of patients with subacute lupus erythematosus and considered them to be virus-like particles and to be diagnostic of this disease. However, they have been described in a wide variety of cell types by numerous workers (*25*), in both man and animals, and their nature remains unknown. Uzman et al. (*25*) suggested a relationship between the tubular arrays and immunoglobulin production, but they gave no indication of their reasons for this consideration.

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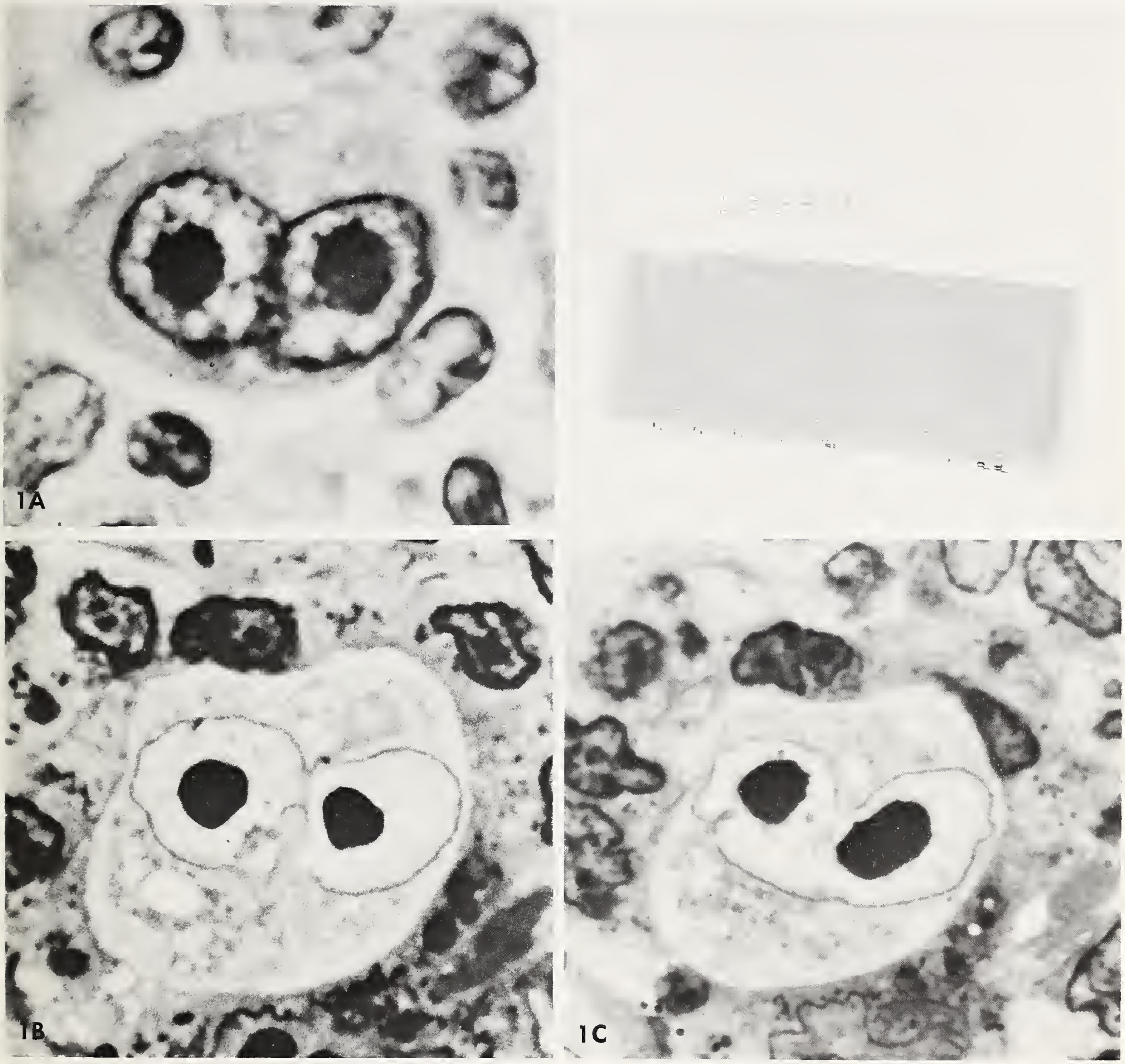

FIGURE 1*A*).—Apparently binucleated, "mirror-image" Reed-Sternberg cell. Formalin-fixed, embedded in paraffin, and stained with hematoxylin and eosin. 1*B*) Paraformaldehyde-glutaraldehyde-fixed tissue embedded in Epon and stained with toluidine blue. In plane of section, cut at 1 mμ, Reed-Sternberg cell appears to have a narrow bridge connecting lobes of nucleus which contains 2 prominent nucleoli. 1*C*) Serial section of same cell seen in figure 1*B* demonstrating clearly that the Reed-Sternberg cell has a single bilobed nucleus containing 2 large nucleoli. × 2600

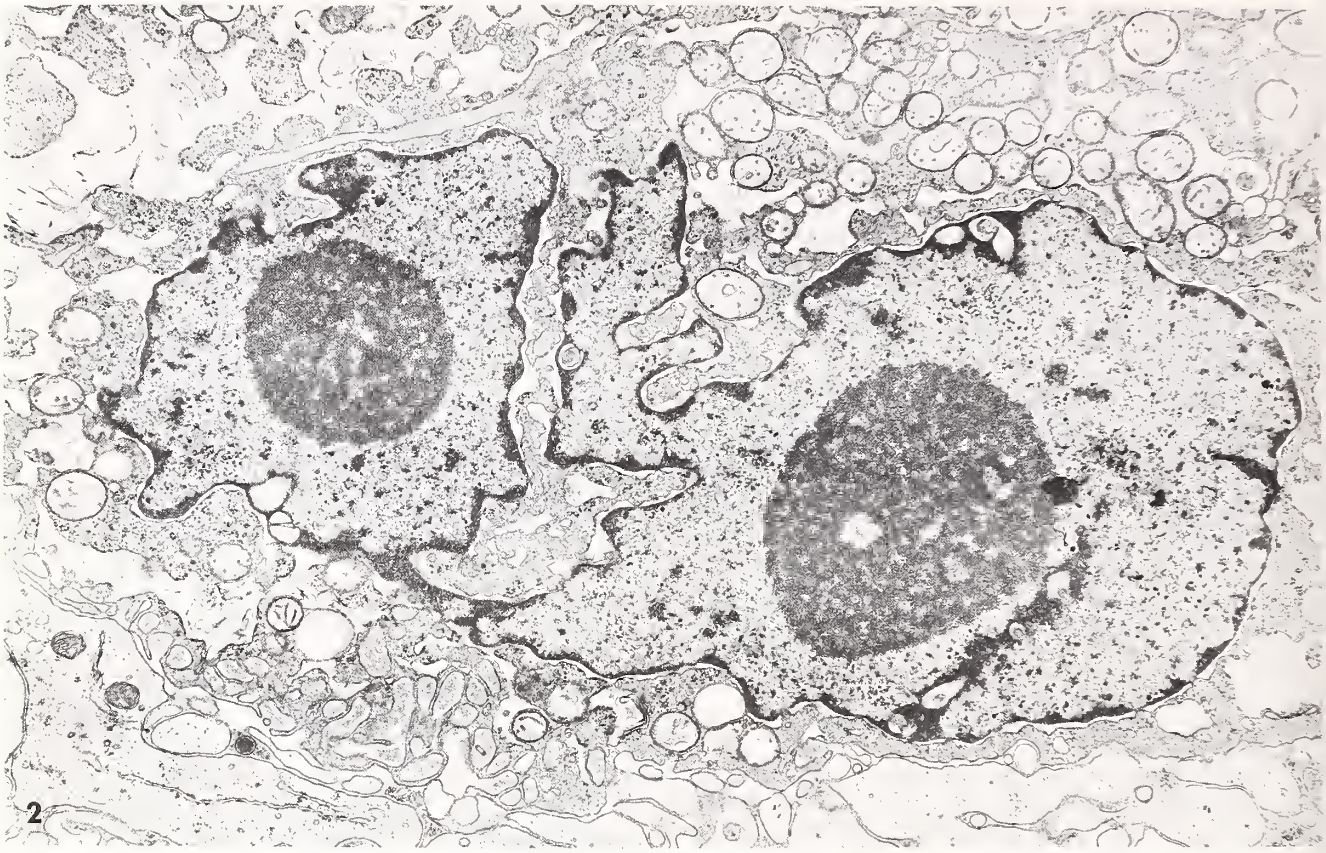

Figure 2.—Electron micrograph of Reed-Sternberg cell selected to demonstrate prominent electron-dense nucleoli within a single nucleus, the lobes of which are connected by a thin bridge. This cell shows degenerative changes by virtue of condensation of chromatin around nuclear membrane, increased density of cytoplasm, and fragmentation of mitochondrial cristae. Such cells are commonly seen in histologic sections. × 3900

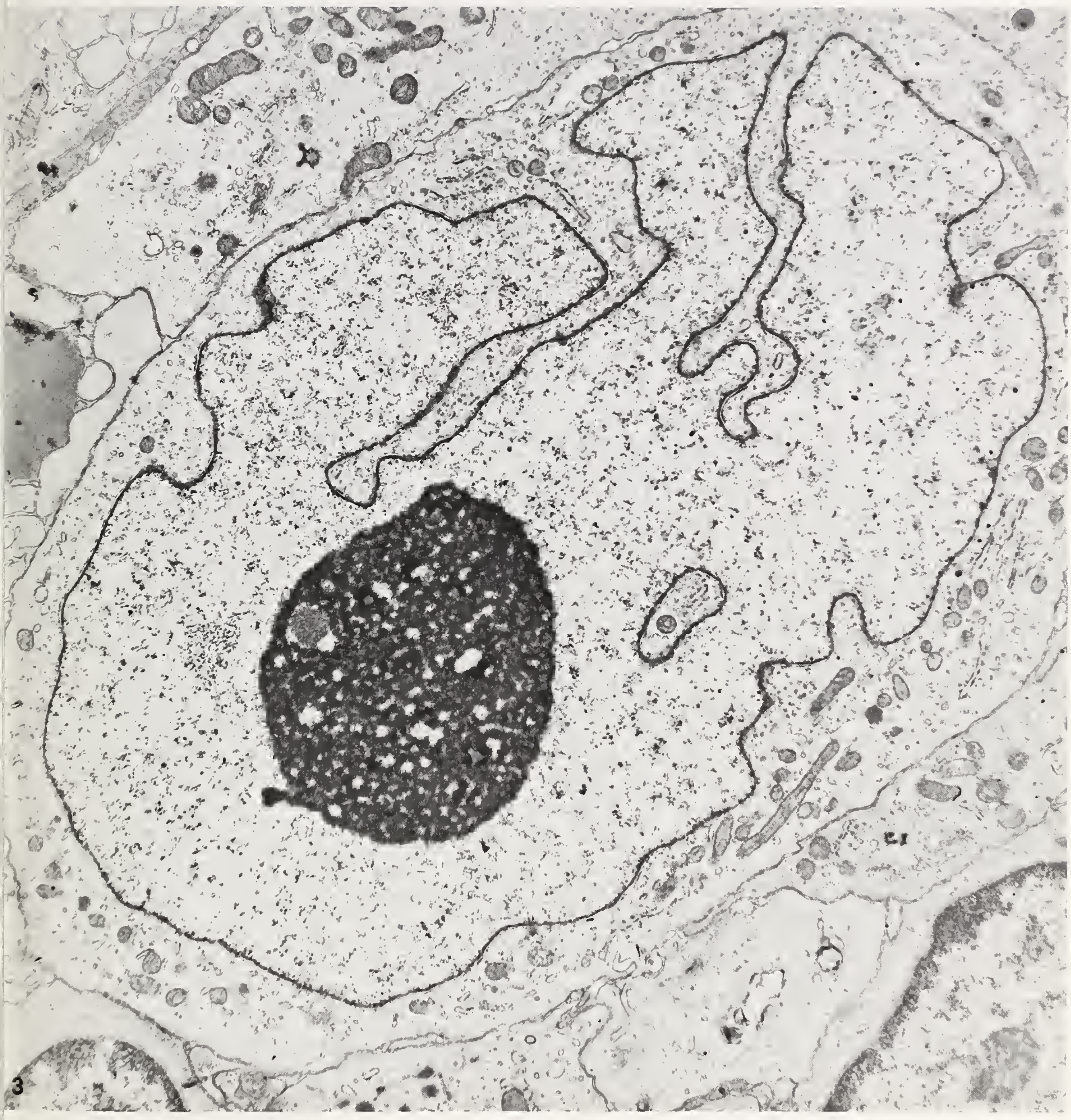

FIGURE 3.—Mononuclear "Hodgkin's cell." Nucleus contains large, circumscribed, electron-dense nucleus. Nuclear membrane is convoluted with cytoplasmic indentations. Nucleoplasm is finely dispersed throughout nucleus. Thin rim of cytoplasm contains free ribosomes and polysomes, sparse rough endoplasmic reticulum, and scattered small mitochondria, some of which are rod-shaped. × 12,000

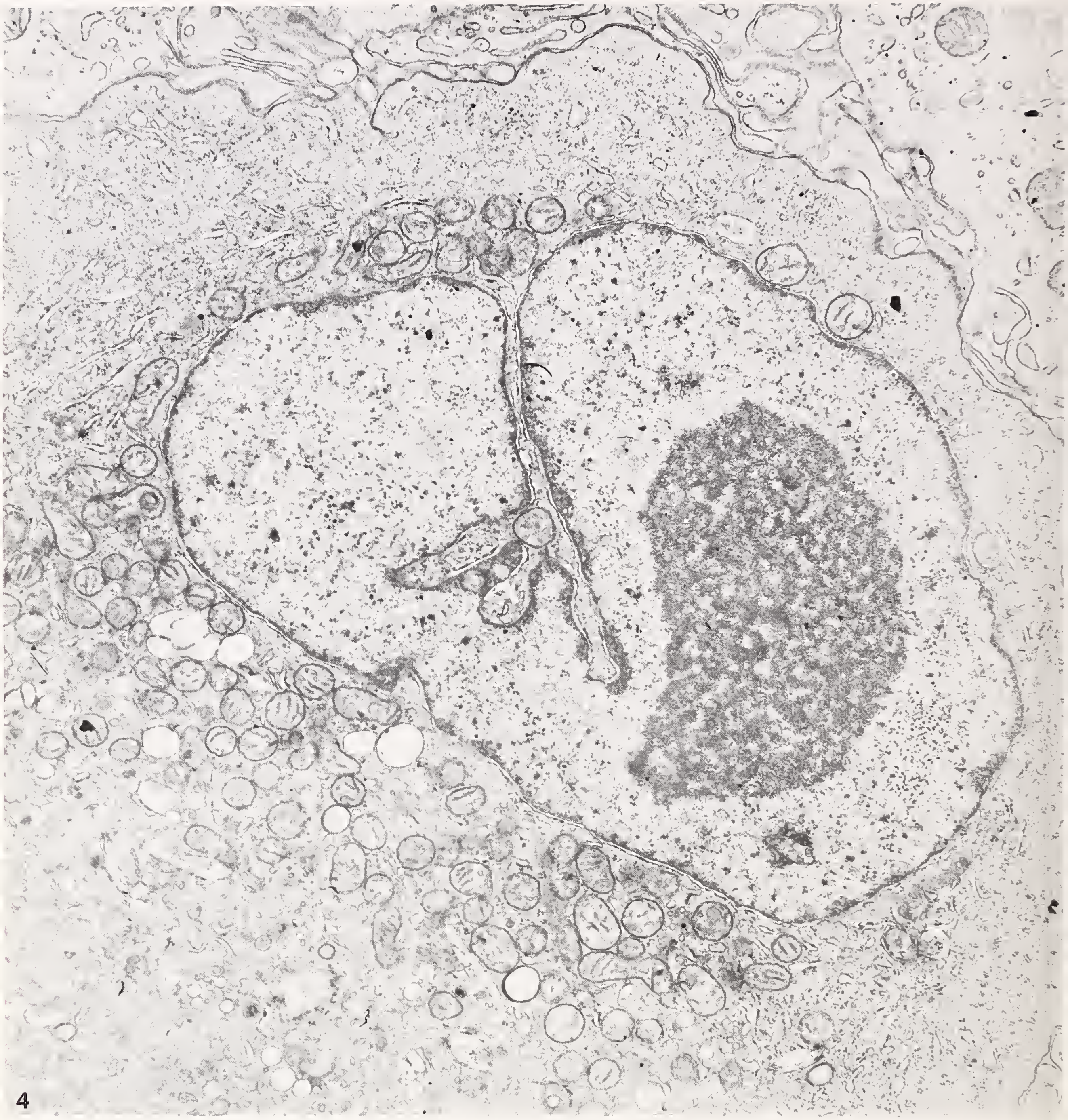

FIGURE 4.—Mononuclear "Hodgkin's cell" with nuclear features similar to those seen in figure 3, but with some condensation of chromatin around nuclear membrane. Deep cleft is seen in nucleus with invagination of cytoplasm. Cytoplasm, however, is more abundant and contains numerous mitochondria together with increased amounts of endoplasmic reticulum. Free ribsomes and polysomes are also prominent. × 21,000

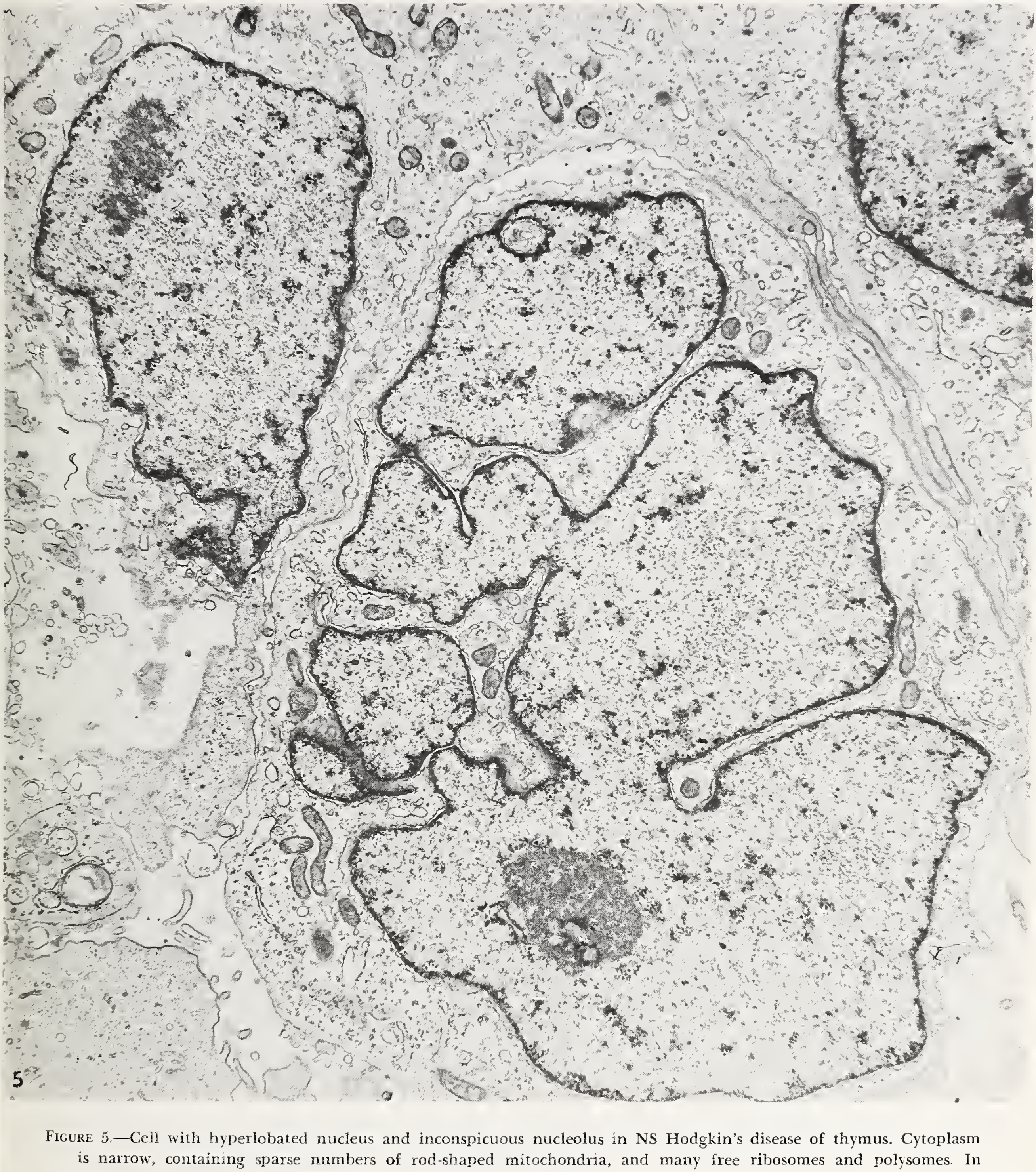

FIGURE 5.—Cell with hyperlobated nucleus and inconspicuous nucleolus in NS Hodgkin's disease of thymus. Cytoplasm is narrow, containing sparse numbers of rod-shaped mitochondria, and many free ribosomes and polysomes. In formalin-fixed tisue, cells of this type appear to lie within 'lacuna-like" spaces. × 12,000

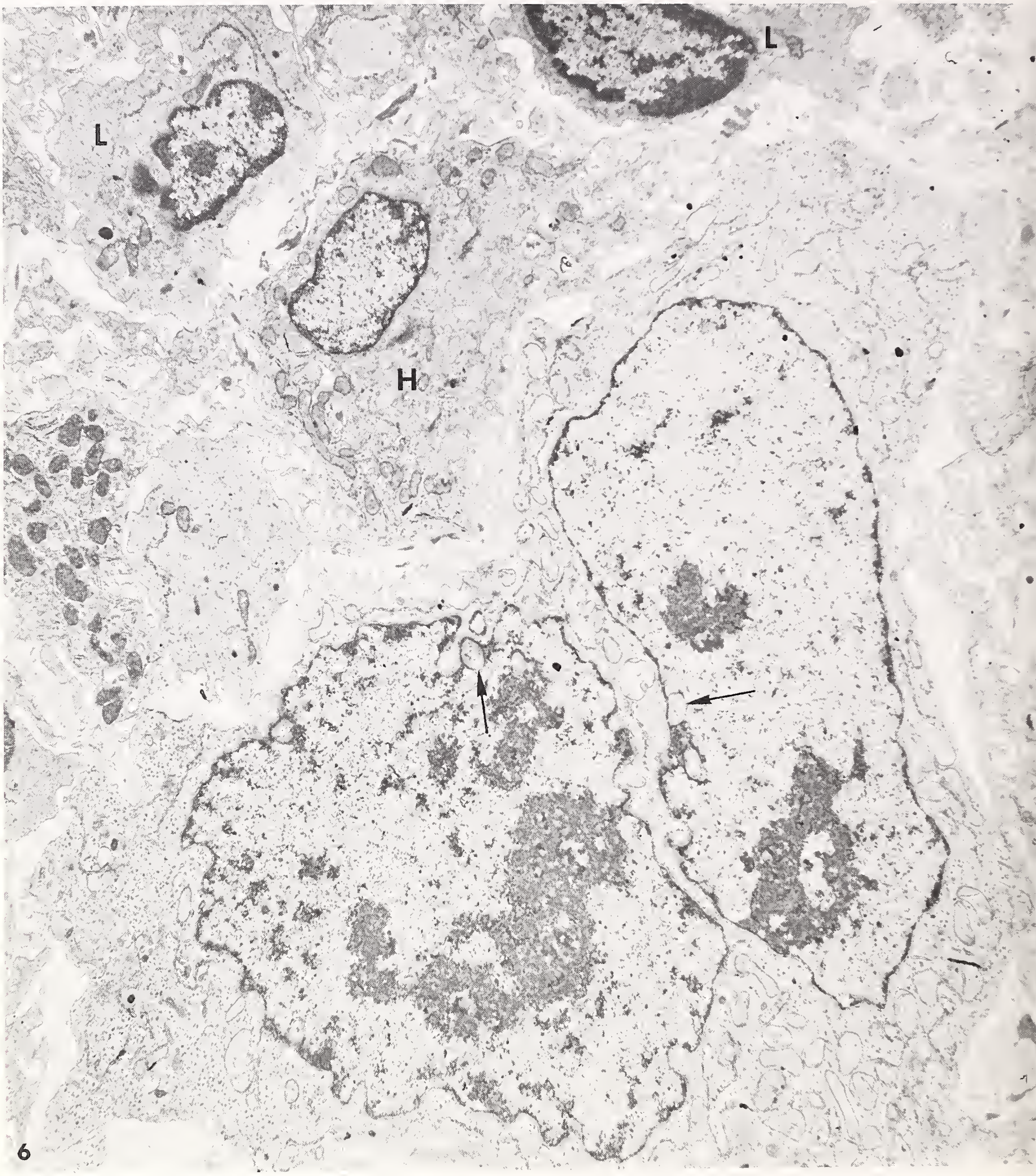

Figure 6.—Complex interdigitating pattern of cytoplasmic membranes of cells in the stroma of Hodgkin's disease. Large binucleated cell on *right* has irregular clumped nuclear chromatin, but is devoid of the characteristic large osmiophilic nucleoli of Reed-Sternberg cell. Cytoplasm contains many free ribosomes and polysomes. Surrounding cells include adjacent histiocyte (H) and 2 lymphocytes (L) with multiple projections of their cytoplasm in form of so-called "uropods." Several nuclear blebs containing cytoplasmic material are visible (*arrows*). × 3900

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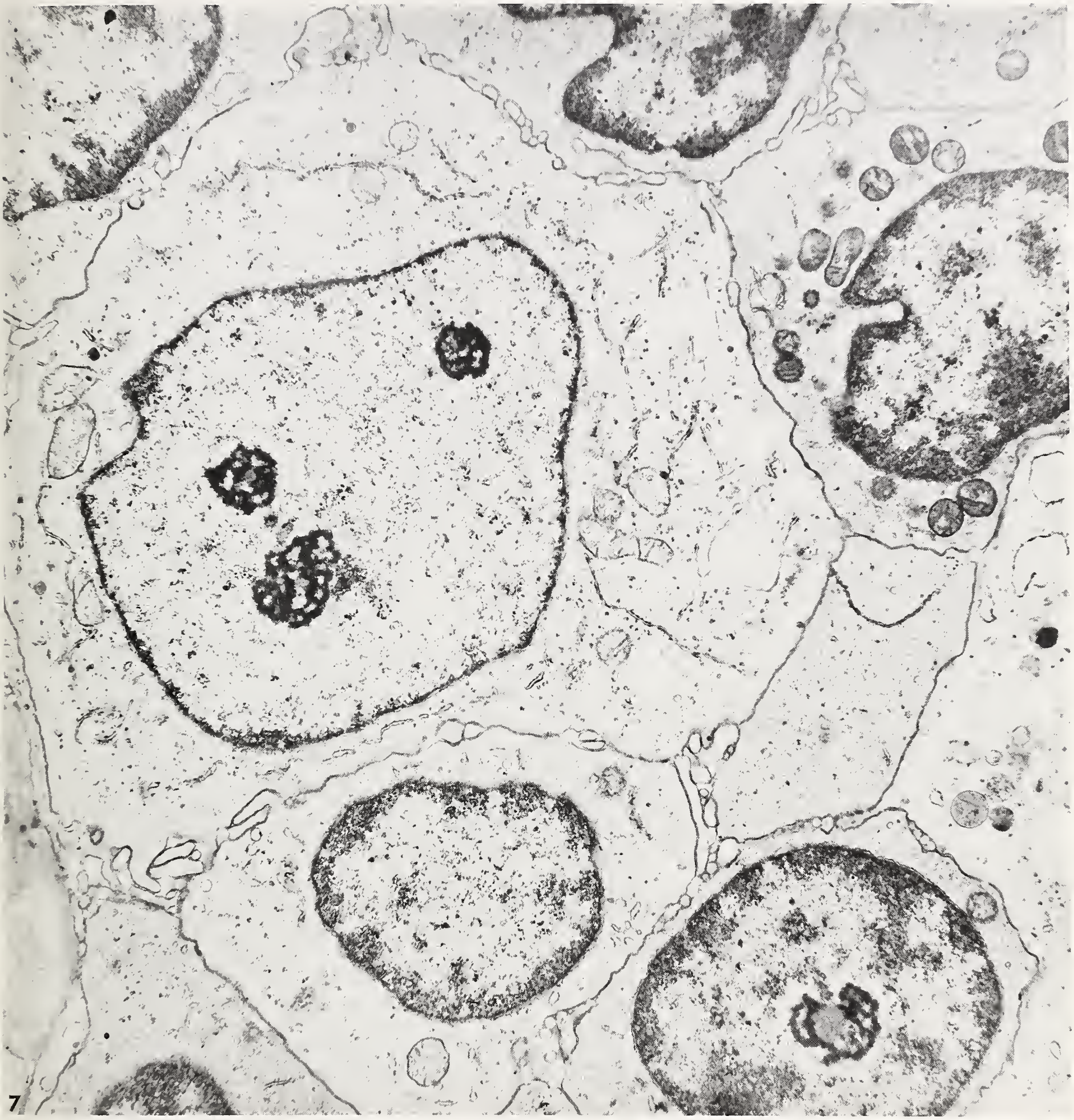

FIGURE 7.—Four normal lymphocytes, three in direct apposition with larger blastlike cell. Nucleoplasm is finely dispersed within enlarged nucleus containing 3 nucleoli. Cytoplasm is increased in amount, containing some smooth and rough endoplasmic reticulum together with many polysomes. × 3900

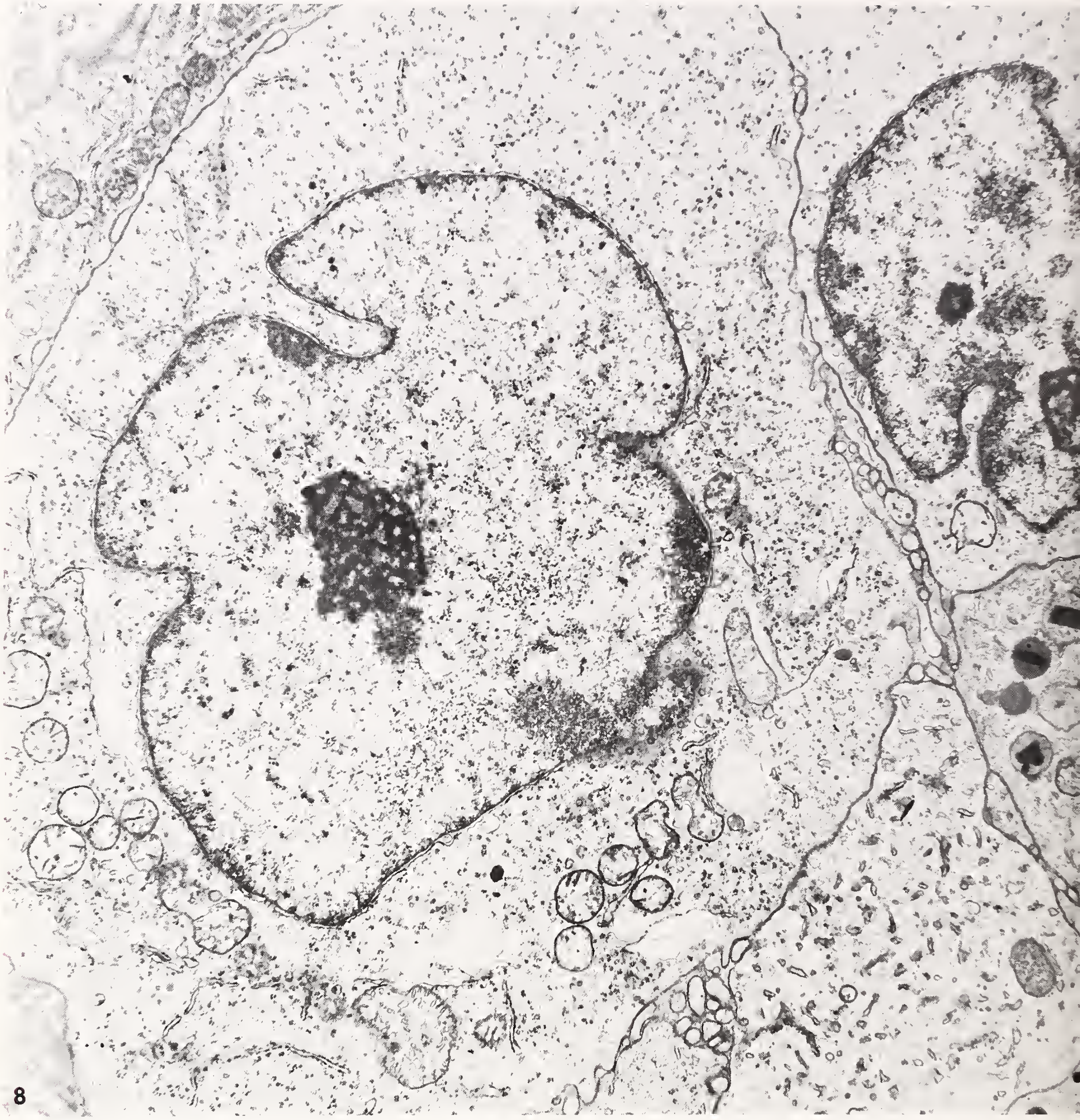

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Figure 8.—Normal lymphocyte and portion of cytoplasm of eosinophilic leukocyte adjacent to blastlike cell resembling transformed lymphocyte. This is characterized by large nucleus, with finely dispersed chromatin and prominent nucleolus. Cytoplasm contains abundant free ribosomes and polysomes. Mitochondria are polarized in region of Golgi apparatus. × 3900

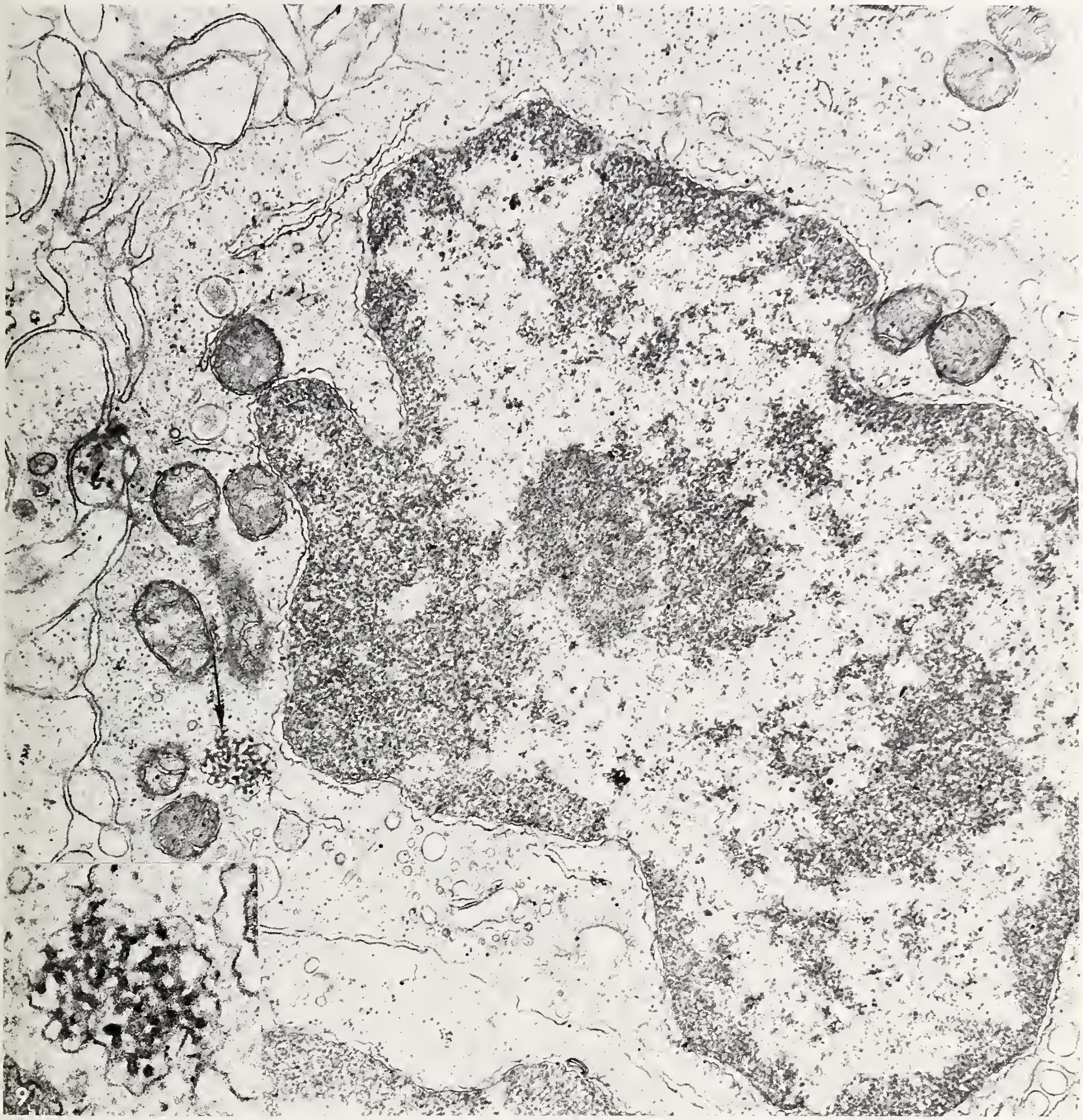

FIGURE 9.—Tubular arrays (*arrow* and *inset*) within the endoplasmic reticulum of a cell with ultrastructural characteristics of lymphocyte (*see* fig. 12). × 30000 (*inset* × 52000)

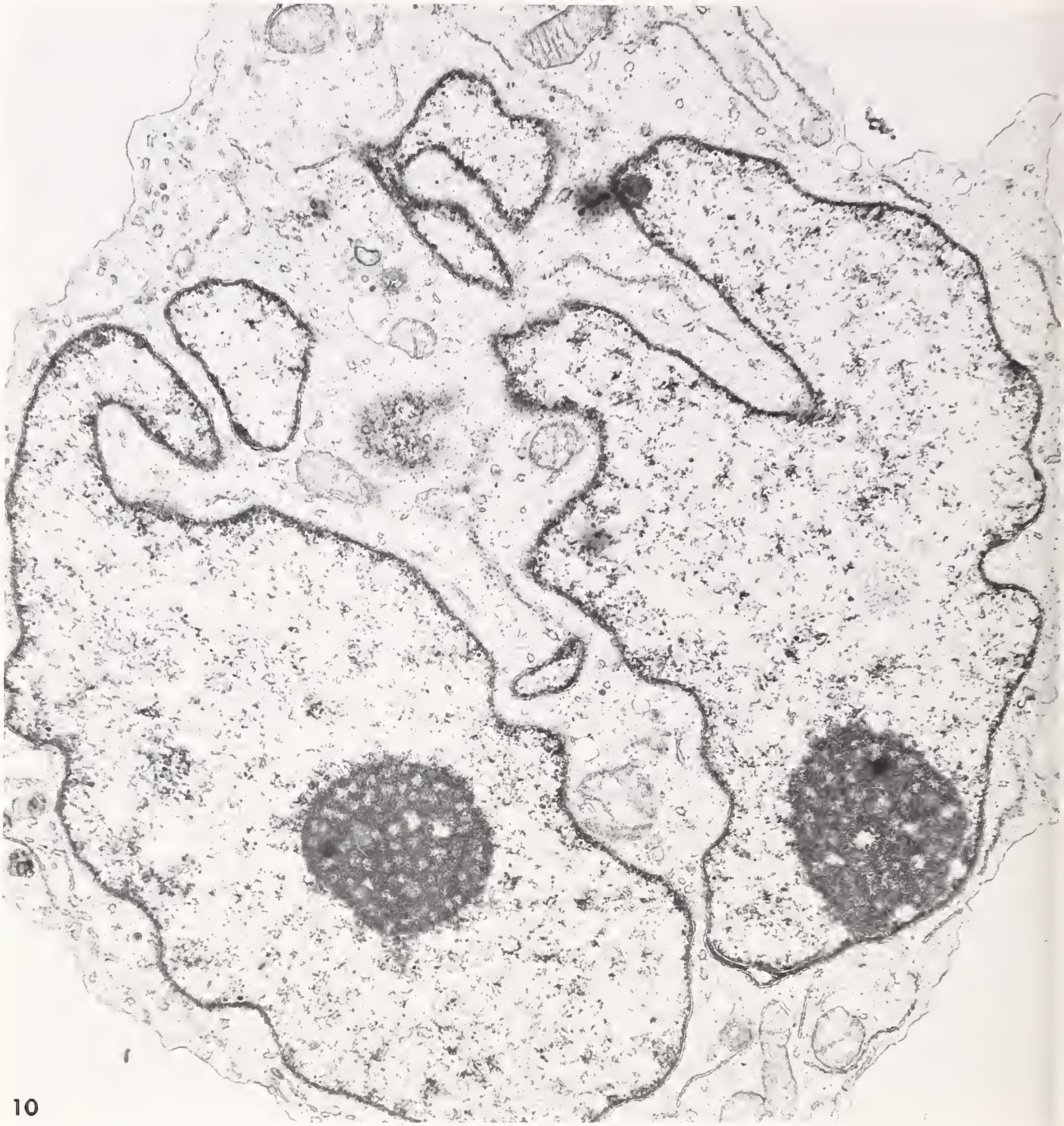

FIGURE 10.—Pellet preparation from splenic lesion established in tissue culture for 5½ months. This cell possesses ultrastructural features resembling those of Reed-Sternberg cell in tissue sections. ×15,000

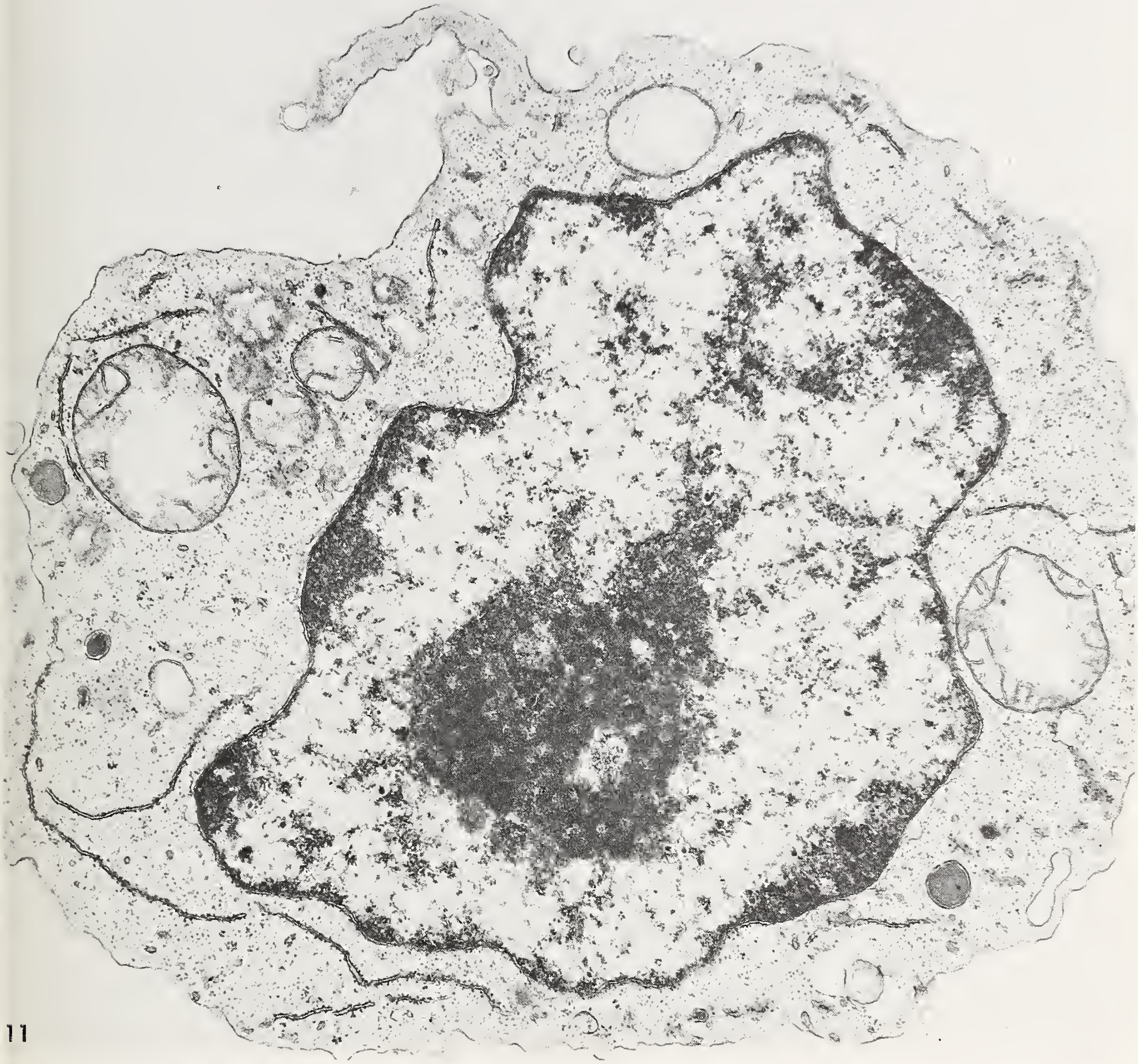

FIGURE 11.—Blastoid cell ultrastructurally resembling transformed lymphocyte, derived from a splenic lesion and established in tissue culture for 5½ months. × 22,500

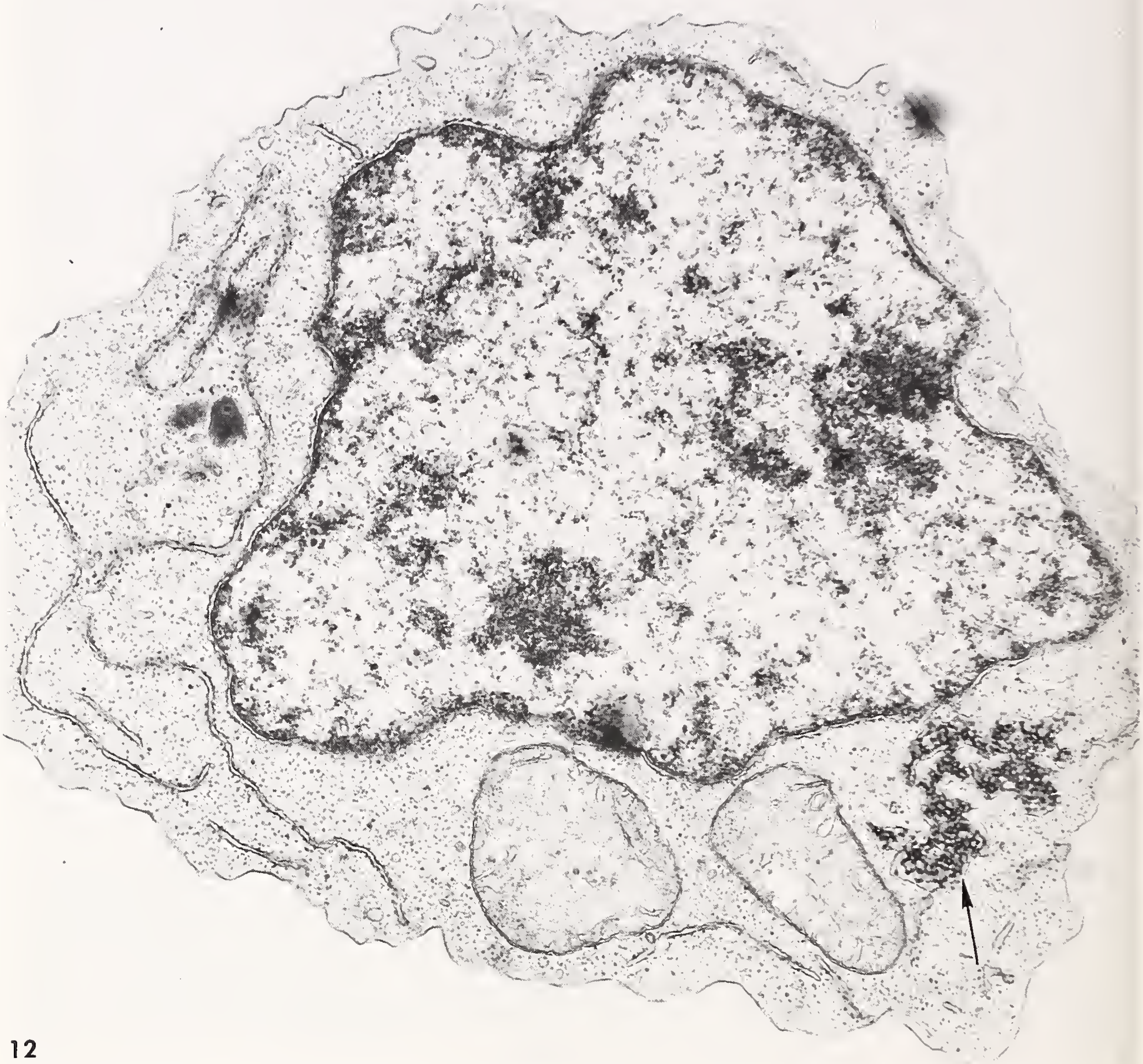

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FIGURE 12.—Tubular arrays (*arrow*) within endoplasmic reticulum of mononuclear cell established in tissue culture for 5½ months and derived from splenic Hodgkin's disease (*see* fig. 9). × 30,000

Quantitative Ultrastructural Analysis of In Vivo Lymphocyte–Reed-Sternberg Cell Interactions in Hodgkin's Disease[1,2]

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SUMMARY—Lymph nodes from 5 untreated patients with the nodular-sclerosis form of Hodgkin's disease were analyzed ultrastructurally for the cellular interaction between lymphocytes and neoplastic cells occurring in vivo. A direct and significant correlation was observed between the tightness of lymphocyte apposition and the ultrastructural cytotoxic changes within neoplastic cells, which supports the concept that lymphocyte immunity against neoplastic cells may be important in the pathogenesis and prognosis of Hodgkin's disease in untreated patients.—Natl Cancer Inst Monogr 36: 239–245, 1973.

PATHOLOGISTS have long noted the infiltration of lymphocytes into human neoplasms and more recently have correlated the degree of such infiltration with the clinical course and prognosis of the neoplasm for individual patients (*1*). Among such human neoplasms, Hodgkin's disease demonstrates a direct correlation between the degree of lymphocyte infiltration and patient survival (*2*). In addition, both the peripheral blood lymphocyte count (*3*) and the degree of reactivity of blood lymphocytes to defined mitogens (*4*) reflect the clinical stage and prognosis of individual patients.

The recognition that many human neoplasms possess tumor-associated antigens (*5*), to which the patient responds with lymphocyte-mediated immunity (*6*), has suggested that the infiltration of lymphocytes into untreated neoplasms may represent an immune response by the patient to the neoplasm, analogous to a homograft reaction (*1*). The recent recognition that tumor-associated antigens may be present in Hodgkin's disease (*7*) has increased the likelihood that lymphocytes may be mediating an immune response against the neoplasm in untreated patients with Hodgkin's disease (*8, 9*).

Lymphocyte immunity against target cells is manifested in vitro as a close apposition of the lymphocytes against the target cells before the target cells are destroyed (*10*) by mechanisms which may utilize lymphocyte plasma-membrane adenosine triphosphatase (*11*). Previous electron microscopic studies of Hodgkin's disease (*12, 13*) did not use fixation techniques, which would have permitted an analysis of interactions between lymphocytes and neoplastic cells; in fact, those studies were largely confined to the ultrastructural analysis of individual Reed-Sternberg cells and Hodgkin's cells (*12, 13*). We recently developed (*9, 14*) quantitative methods for an ultrastructural analysis of cell interactions in lymph nodes more adequately fixed for the preservation of in vivo structure than were those previously available (*12, 13*).

MATERIALS AND METHODS

All 5 study patients sampled were previously untreated and had a histologic diagnosis of

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported in part by Public Health Service grants CA10174 from the National Cancer Institute and AM01006 from the National Institute of Arthritis and Metabolic Diseases; by grant IC–45 from the American Cancer Society, Inc.; by a Research Scholar Award from the Leukemia Society to Dr. Frenster, to whom requests for reprints should be sent.

Hodgkin's disease, nodular-sclerosis (NS) type, made in the Division of Surgical Pathology. All patients had Stage III disease following complete pathologic staging (*15*), all were free of constitutional symptoms, and all had peripheral blood lymphocyte counts $>1500/\text{mm}^3$. Biopsies of lymph nodes in such patients were made for diagnostic staging, and those selected for the current study were confined to lymph nodes not previously seen by lymphangiography (*16*). All lymph nodes were subsequently confirmed as positive for Hodgkin's disease, NS type, by concurrent thick-section light microscopy (*16*).

Random samples of each lymph node were placed, within 30 seconds of excision by the surgeon, into ice-cold Eagle's minimal essential medium (Gibco), at pH 7.4, containing 5% glutaraldehyde, and were then brought to the laboratory where they were sectioned into 1 mm^3 cubes. These cubes were then postfixed in 1% OsO_4, dehydrated in ethanol, embedded in Epon, sectioned at 0.1μ thickness, stained with 5% uranyl acetate, and examined at 80 kv under high resolution in a Siemens 1A electron microscope as previously described (*17*).

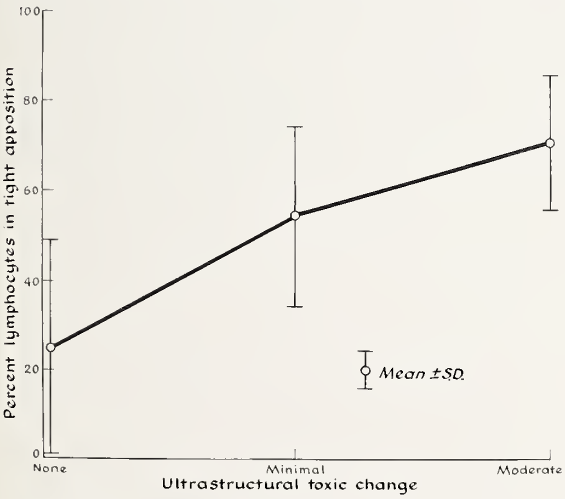
The ultrastructural classification of Bernhard and Leplus (*12*) was used to identify neoplastic polynuclear Reed-Sternberg cells, mononuclear Reed-Sternberg cells, and mononuclear Hodgkin's cells (*12*). Each of these neoplastic cells was coded and imaged on a projector plate at 10,000 magnification. Five prints of each cell were then prepared with an additional fourfold magnification. One print was employed by an independent observer to identify the neoplastic cell type. A second print was used by an independent observer to identify all non-neoplastic cells. A third print was utilized for a quantitative sector analysis (*9, 14*) of each neoplastic cell (outlined in black ink); a Lietz map reader was used to yield neoplastic cell circumference values in mm. Each surrounding cell apposing the neoplastic cell was assigned a sector of the neoplastic cell circumference. This sector was measured in turn by the map reader; the measured sector length was then expressed as a decimal fraction of the total neoplastic cell circumference (*9, 14*). The surrounding apposed non-neoplastic cells were identified as either lymphocytes, macrophages, fibroblasts, plasma cells, eosinophils, neutrophils, or endothelial cells (*18*) and were then identified by an independent observer as in either tight or loose apposition to the neoplastic cell (*9, 14*). The sectors of lymphocytes in tight apposition to a single neoplastic cell were then summed as a total decimal fraction of the neoplastic cell circumference for each neoplastic cell. A fourth print was utilized by an independent observer to estimate the degree of ultrastructural cytotoxic change within each neoplastic cell, with special attention given to mitochondrial swelling, nuclear edema, disruption of the endoplasmic reticulum, and irregularities of the plasma membrane (*19*). Finally, the degree of such neoplastic cell cytotoxicity was correlated with the decimal fraction of the cell circumference occupied by the lymphocytes in tight apposition to the particular neoplastic cell.

RESULTS

Random sampling of lymph nodes from untreated patients with Hodgkin's disease, NS type, revealed all cell types as originally described by Bernhard and Leplus (*12*). In particular, polynuclear Reed-Sternberg cells (fig. 1A), mononuclear Reed-Sternberg cells (fig. 1B), and mononuclear Hodgkin's cells (fig. 1C) were found in an approximate ratio of 1:10:100 and comprised 10–15% of all cells in the sampled lymph nodes. Cells in the neoplastic series were also observed in the various stages of cell division (fig. 1D), often in the process of forming binucleate cells via nuclear division without cytoplasmic division (fig. 1D).

Lymphocytes comprised approximately 75% of all cells in the sampled lymph nodes and were either of the small or medium-sized type (*20*), with single regular nuclei, cytoplasmic monosomes, scanty endoplasmic reticulum, and no evidence of nuclear activation (*21*). Macrophages, fibroblasts, plasma cells, eosinophils, and endothelial cells, as observed in sampled lymph nodes, will be described elsewhere (*22*). Lymphocytes were frequently found in parallel orientation to the surface of neoplastic cells (figs. 1A, 1B) and sometimes were seen deeply invading those neoplastic cells displaying moderate degrees of cytotoxic change (fig. 1C). Uropod formations on lymphocytes were occasionally observed to be directed toward neoplastic cells.

The first 25 neoplastic cells collected by random sampling in equal numbers from each of the 5 study patients in each of the categories, "no, minimal, or moderate degrees of ultrastructural cytotoxic change," respectively, were correlated with the decimal fraction of their total cell circumference occupied by lymphocytes in tight apposition. A positive direct correlation was noted (text-fig. 1) between these 2 cellular parameters which was significant at the $P=0.01$ level by the t test of the means of samples from the no-toxicity and moderate-toxicity groups. The relative proportions of polynuclear and mononuclear Reed-Sternberg cells and Hodgkin's cells were approximately equal among the categories of no, minimal, and moderate degrees of ultrastructural cytotoxic change, and the 5 study patients did not vary significantly among themselves in either of these parameters. The effects of macrophage apposition on the neoplastic cell have been analyzed separately (*22*).



TEXT-FIGURE 1.—Relationship of tight lymphocyte apposition to ultrastructural cytotoxic change occurring within 25 neoplastic cells in each of the categories of no, minimal, and moderate degrees of such toxic change (*see* text).

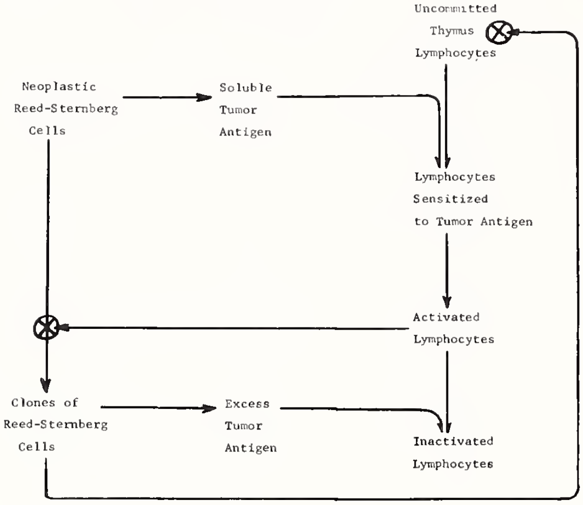
DISCUSSION

By use of rapid fixation in cold solutions of glutaraldehyde in the operating room, the quality of electron micrographs of sampled lymph nodes from untreated patients with Hodgkin's disease has improved, compared to previous studies in which only osmic acid fixation was used (*12, 13*). This improvement has permitted the analysis of lymphocyte and macrophage apposition to neoplastic cells in Hodgkin's disease (*9, 14*) and has revealed a significant correlation between tight apposition by lymphocytes to neoplastic cells and ultrastructural cytotoxic changes within such neoplastic cells (text-fig. 1).

It is not yet apparent whether lymphocyte tight apposition is an independent or a dependent variable in the cellular interaction, or whether such apposition plays a causal role in the observed ultrastructural cytotoxicity within the neoplastic cells. However, within in vitro systems, tight apposition by immune lymphocytes is a prerequisite for the expression of cytotoxicity within target cells to which the lymphocytes have been sensitized (*10*). Such cytotoxicity may be mediated via the increased levels of lymphocyte plasma membrane adenosine triphosphatase, which is found in immune lymphocytes when they are exposed to target cells to which they are sensitized (*11*).

Lymphocytes in the peripheral blood of untreated patients with Hodgkin's disease often are larger and more active in DNA synthesis than normal (*8*), but late in the course of the disease they often decrease in number (*3*) and become less responsive to defined mitogens such as phytohemagglutinin (*4*). Advanced neoplasms in man are associated with increased levels of free-circulating tumor antigen (*23*). Such free antigen in the presence of small quantities of antibody can decrease the activity of lymphocytes specifically sensitized to the antigen (*24*) and may account for the loss of lymphocyte reactivity in advanced Hodgkin's disease (*3, 4*). Decreases in peripheral blood lymphocyte counts in advanced Hodgkin's disease may relate to direct invasion by neoplastic cells of such vital lymphopoietic organs as the thymus and the spleen. These relationships between neoplastic cell proliferation, increasing quantities of tumor-associated antigen, and lymphocyte immunity and inactivation are summarized in text-figure 2 and are supported by the viral transformation hypothesis of Order and Hellman (*25*). A critical test of this relationship will be an assay of the effects of isolated Hodgkin's tumor antigen (*7, 25*) on lymphocytes from patients with minimal or advanced Hodgkin's disease in which a mitogenic response by the lymphocytes might be expected (*26*) in the absence of significant quantities of antibody in the in vitro test system (*24*).

Confirmation of the role of antigen, antibody, and lymphocytes in the pathogenesis and prognosis of Hodgkin's disease would prompt consideration of such therapeutic manipulations as nonspecific T-lymphocyte activation (*1*), plasmapheresis of circulating antigens, and immunosuppression of B-lymphocytes as a source of inactivating antibody (*1*).



TEXT-FIGURE 2. — Possible lymphocyte–Reed-

TEXT-FIGURE 2. — Possible lymphocyte–Reed-Sternberg cell interactions in Hodgkin's disease. Neoplastic cells may possess a tumor-associated antigen (*see* text) which can sensitize thymus-dependent T-lymphocytes. Such sensitized lymphocytes, when activated, can antagonize the proliferation of the neoplastic cells by inducing cytotoxic changes within such cells (*see* text). Should immunosuppression of the lymphocytes or antigen deletion of the neoplastic cells occur, neoplastic cell proliferation could progress with the production of excessive quantities of soluble tumor antigen and the resultant inactivation of sensitized lymphocytes (*see* text). Neoplastic cell proliferation can also decrease T-lymphocyte counts by the direct invasion of such lymphopoietic organs as the thymus and the spleen (*see* text).

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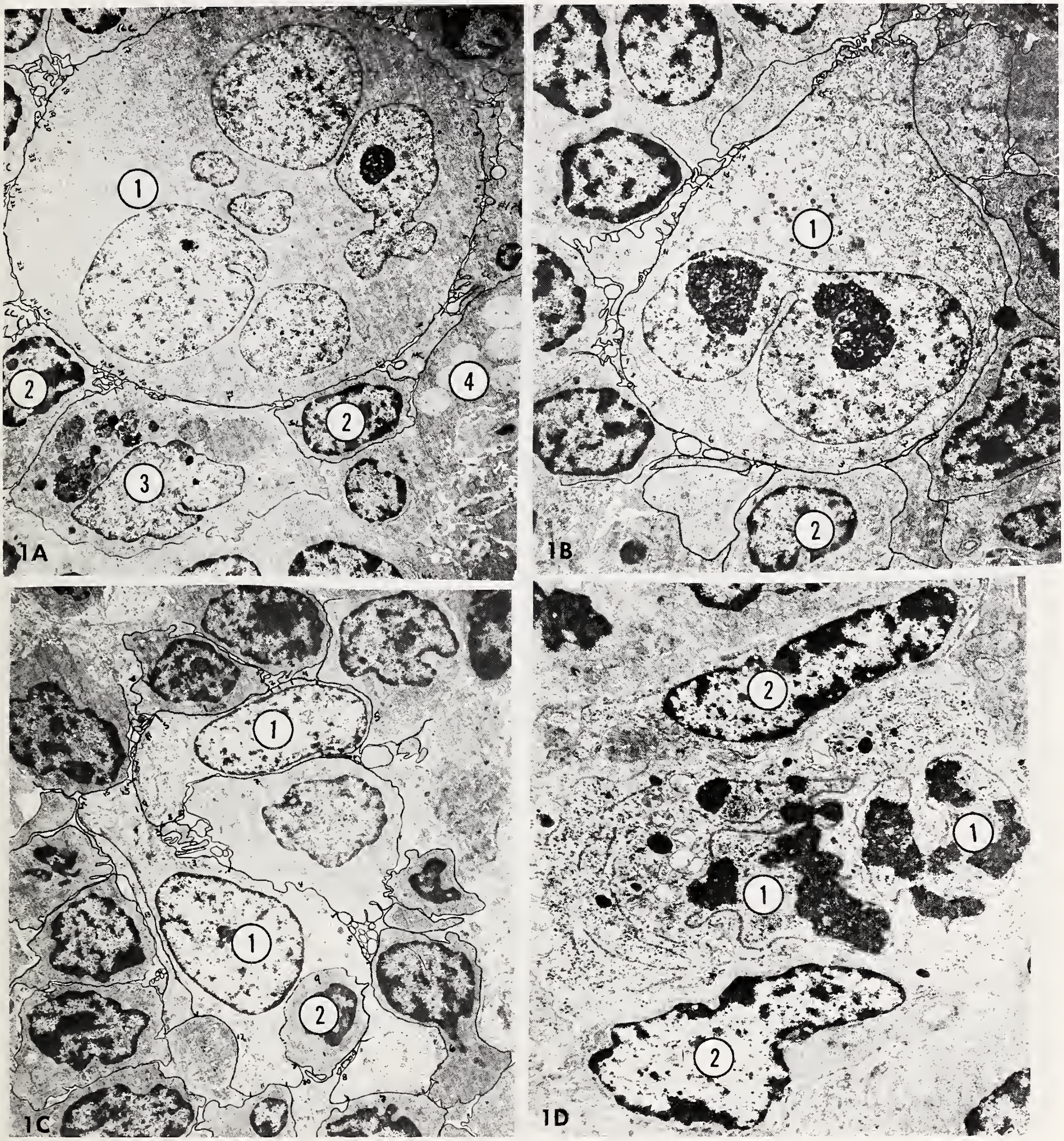
Figure 1A.—Polynuclear Reed-Sternberg cell (1), surrounded by small lymphocytes (2), a macrophage (3), and a fibroblast (4). Neoplastic cell shows no significant ultrastructural cytotoxic change, and surrounding cells are all in loose apposition. Neoplastic cell (*outlined in black ink*) and sectors of surrounding cells numbered from 1 to 32. × 2000

Figure 1B.—Mononuclear Reed-Sternberg cell (1) with deeply hyperlobated nucleus, hypertrophic nucleoli and surrounding lymphocytes (2), some of which orient themselves to the circumference of the neoplastic cell. Neoplastic cell shows a minimal degree of cytotoxic change (slight nuclear edema); surrounding lymphocytes are in moderately tight apposition. × 3000

Figure 1C.—Two mononuclear Hodgkin's cells (1 and 1), with a deeply invading small lymphocyte (2). Neoplastic cells show moderate degrees of cytotoxicity (nuclear and cytoplasmic edema), whereas surrounding lymphocytes are in tight apposition or are actually invasive. × 3000

Figure 1D.—Mitotic neoplastic cell, in late telophase with complete reconstitution of the nuclear membranes (pentalaminar) of both daughter nuclei (1 and 1), but with no indication of cytoplasmic division, suggesting the formation of a binucleated cell. Surrounding medium-sized lymphocytes are in loose apposition. × 4000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Peripheral and Central Lymph in Hodgkin's Disease: Preliminary Report[1]

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SUMMARY—The cell output from catheterized peripheral lymphatics was followed in 25 patients during 2–6 days. Six of the patients had Hodgkin's disease, eleven had chronic lymphocytic leukemia or lymphosarcoma, and eight had solid tumors. Our early studies of thoracic-duct lymph cytology in Hodgkin's disease are also discussed. The 3 groups averaged around 100–200 cells/mm^3 of the peripheral lymph with about 80–90% lymphocytes. Differential counts revealed somewhat fewer lymphoid cells and more macrophages in the Hodgkin's disease group than in the other patients. In the Hodgkin's disease group, the lymphocyte number in peripheral and central lymph correlated with the lymphocyte level of peripheral blood. The number of B-lymphocytes was elevated. There was $<$ 1% B-lymphocytes in the peripheral lymph in the Hodgkin's disease and patients with solid tumors. The frequency was somewhat higher in the leukemic group with variations from $<$ 1 to 50%. Thoracic-duct lymph examination in 15 patients with Hodgkin's disease revealed that abdominal node involvement increased the number of lymphoblasts in the lymph and also the number of macrophages and histiocytes. Reed-Sternberg cells and Hodgkin's cells were found in the thoracic-duct lymph in all patients, with involvement of the para-aortic nodes above the L IV level. Kinetics of cell migration and mechanisms of the dissemination of Hodgkin's disease are discussed.—Natl Cancer Inst Monogr 36: 247–252, 1973.

IN MAN and mammals, there is a large-scale recirculation of lymphocytes between blood and lymphoid tissue through the walls of the post-capillary venules (*1*). The thymus-derived lymphocytes (T-cells) recirculate to a greater extent than the plasma cell precursors (B-cells) (*2*). A small proportion of lymphoid cells migrate also into nonlymphoid tissue, e.g., subcutis (*3*). Their route back to the blood is through the peripheral lymphatics.

In lymphomas, the lymphocyte recirculation may be altered. In chronic lymphocytic leukemia (CLL), there is some evidence of disturbances revealed by thoracic-duct cannulation (*4–6*). In Hodgkin's disease, the cellularity of thoracic-duct lymph has been reported (*7, 8*), but recirculation has not been studied.

In the present study, the cellularity of the peripheral lymph—which has not yet passed through any lymph node—has been investigated in CLL and in Hodgkin's disease to examine the circulation of cells from nonlymphoid tissue. Some very preliminary results will be presented and also the main findings from earlier thoracic-duct lymph examination in Hodgkin's disease (*7, 8*). Kinetics of cell migration in patients with Hodgkin's disease and possible mechanisms of dissemination of the disease will be discussed.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

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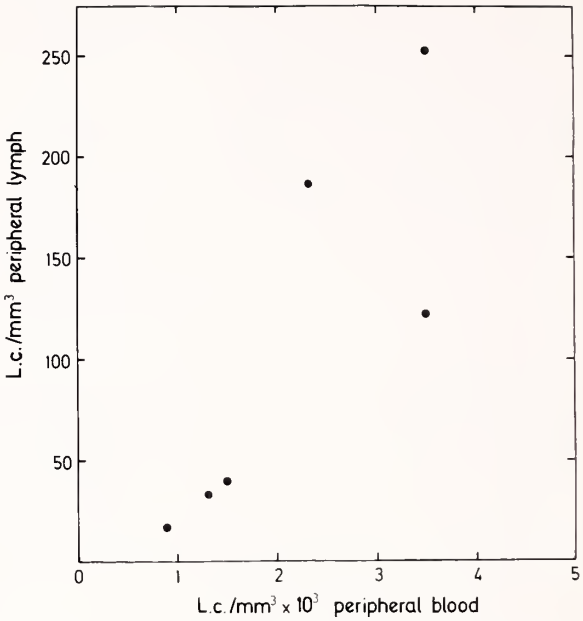
MATERIALS AND METHODS

Peripheral lymph.—Twenty-five patients with untreated malignancies were successfully cannulated. Eight had solid tumors. All were in good condition; this group served as controls. Eleven patients had CLL or lymphosarcoma with leukemia, and six had Hodgkin's disease. A subcutaneous lymphatic on the leg was cannulated with a siliconized polyethylene tube (*9*). Lymph was collected for 2–6 days in a glass tube taped to the leg, containing 40 IU heparin in saline. Samples were removed 3 or 4 times a day for measurements of lymph flow and cell number and for cytologic examination. The final blood and lymph samples were examined for B-lymphocytes by immunofluorescence, with the use of membrane Ig as a marker of human B-lymphocytes (*10*). The lymph flow and the white blood cell counts presented were the means of all observations for each patient. Differential counts were performed on 1,000 cells from each of the Hodgkin's disease patients from 1 sample collected in the middle of the cannulation period.

Thoracic-duct lymph.—Of 15 patients with Hodgkin's disease who were successfully cannulated, 11 had intra-abdominal lymph-node involvement diagnosed by lymphography. Laparotomy was not performed. Methodology and results were published (*7, 8*). Lymph was collected for 1–3 days. The output of the various cell types in the thoracic-duct lymph was estimated by differential counts or by counts of Hodgkin's and Reed-Sternberg cells on Millipore filters after filtration of 3 ml lymph.

RESULTS

Peripheral Lymph



TEXT-FIGURE 1.—Peripheral lymph in Hodgkin's disease. Cell number in peripheral lymph in relation to lymphocyte number in peripheral blood.

Table 1 gives the lymph flow, cell counts, and differential counts in the 3 groups. The cell designated "other cells" are granulocytes and cells that could not be identified. In general, the cell output per hour varied only slightly throughout the cannulation period. The 3 groups did not differ significantly; there was a tendency toward a low lymphocyte number in the Hodgkin's disease patients, who also had a high percentage of macrophages. Table 2 gives details of the Hodgkin's disease patients, all but 2 of whom had extensive disease. There is a considerable variation from patient to patient in lymphocyte number in blood and lymph, being lowest in the patients with extended disease (table 2). Text-figure 1 shows that the lymphocyte number in peripheral blood correlated with that in the lymph. The macrophages comprised 6–25% of the cells in the lymph, the percentage being highest in the lymphopenic patients. The percentage of monocytes in peripheral blood and peripheral lymph did not correlate.

The percentage of B-lymphocytes was evaluated in 4 each of the cancer and Hodgkin's disease patients and in 7 of the leukemia patients. In the Hodgkin's disease group, 7–10% of the blood lymphocytes were B-cells (table 2). No B-lymphocytes could be found in the peripheral lymph in this group nor in 3 of the cancer patients. One of the cancer patients had some B-cells in the lymph—<1%. Of the blood lymphocytes, 74% were B-cells in this patient (normal level about 10% in our laboratory).[5]

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[5] The Institute of Immunology and Rheumatology, Rikshospitalet.

TABLE 1.—*Flow rate and cell content of peripheral lymph*

Diagnosis	Number of patients	Flow rate (ml/hr)	Cells/mm^3	Percentage distribution		
				Lymphocytes	Phagocytes†	Other cells
Carcinoma	8	0.8	163 ± 53*	83 ± 2.6*	9.4 ± 1.8*	8
CLL or lymphosarcoma	11	0.6	225 ± 67*	86 ± 4.2*	7.3 ± 1.7*	8
Hodgkin's disease	6	0.8	134 ± 42*	76 ± 4.9*	17.0 ± 3.4*	8

* Standard error of the mean.
† Includes monocytes.

TABLE 2.—*Peripheral lymph in Hodgkin's disease*

		Peripheral lymph						Blood	
				Percentage distribution of cells					
Case No.	Stage	Flow rate (ml/hr)	Cells/mm^3	Lymphocytes	B-lymphocytes	Macrophages*	Others	Lymphocytes/mm^3 × 10^3	B-lymphocytes (%)
26	II-A†	0.4	179	68	—	25	8	3.5	—
27	IV-B	0.8	54	61	<1	22	17	1.3	—
31	IV-B	0.7	55	76	—	9	15	1.5	7
34	III-A†	0.2	216	87	—	13	0	2.3	—
40	I-A	2.0	276	93	<1	6	1	3.5	10
41	IV-B	0.8	24	72	<1	25	3	0.9	10
Mean		0.8	134	76	<1	17	8	2.2	
Standard error of mean		0.26	42	4.9		3.4		0.46	

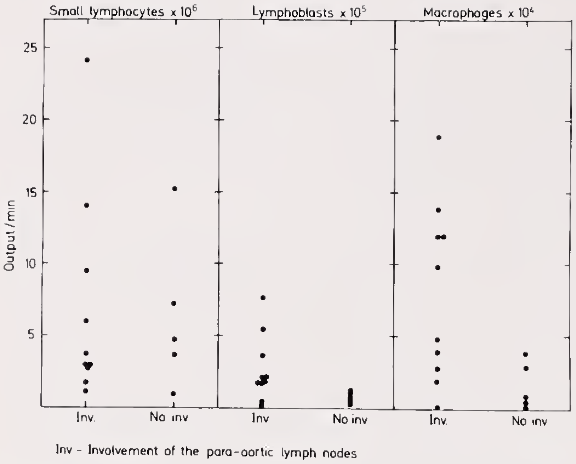
* Includes monocytes.
† Clinical stage.

B-lymphocytes comprised >90% of the blood lymphocytes in 6 leukemia patients and 10% in 1 leukemia patient. In the peripheral lymph, the frequency was 0–5%, except for 1 patient in whom it was 50% of the lymphoid cells.

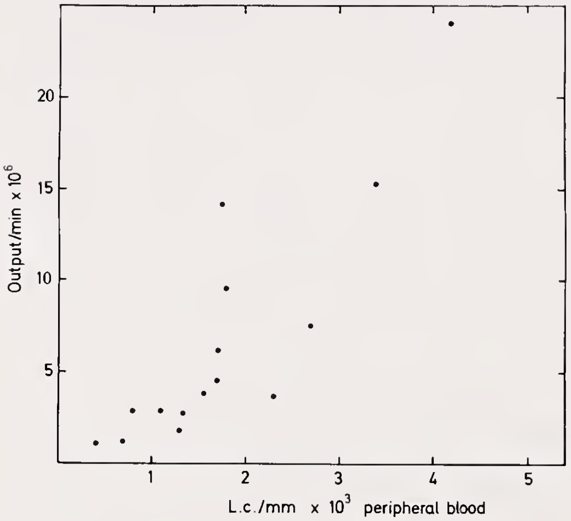
Thoracic-Duct Lymph

The output of lymphoid cells and monocytes related to involvement of the para-aortic lymph nodes is given in text-figure 2, and the range of output of different types of cells in table 3. There was no absolute correlation between para-aortic lymph-node involvement and the output of small lymphocytes in the thoracic duct (text-fig. 2). Histiocytes, monocytes, and macrophages, however, were more frequent in the lymph of patients with involvement of the para-aortic nodes, and the percentage of large basophilic lymphocytes was higher in this group than in those patients whose nodes were apparently free of disease (text-fig. 2). There was a clear correlation between the lymphocyte number in peripheral blood and the output of lymphocytes in the thoracic duct (text-fig. 3).

Hodgkin's and Reed-Sternberg cells were found in 10 of 15 patients (table 4). The output of Hodgkin's cells was always higher than that of Reed-Sternberg cells. The highest number measured was 16 cells per minute in a 20-minute period (table 3). In the other patients, the maximum output of these cells was much lower, between 1 and 3 cells per minute. Hodgkin's and Reed-Sternberg cells were observed in 8 of the patients with para-aortic lymph-node involvement (table 4). The para-aortic lymph nodes cranial to L IV were involved in all of these. Three patients with intra-abdominal lymph-node involvement did not show Hodgkin's or Reed-Sternberg cells in the lymph. The lymph nodes involved in these patients were in the pelvic or para-aortic area caudal to L IV (table 4).



TEXT-FIGURE 2.—Thoracic-duct output per minute of small lymphocytes, lymphoblasts, and macrophages related to para-aortic lymph-node involvement in 15 patients with Hodgkin's disease [from Engeset et al. (*8*)].



TEXT-FIGURE 3.—Thoracic-duct lymph in Hodgkin's disease. Output of small lymphocytes per minute in relation to lymphocyte number in peripheral blood [from Engeset et al. (*8*)].

TABLE 3.—*Thoracic-duct lymph output of cells per minute in patients with Hodgkin's disease* [from Engeset et al. (*8*)]

Cell type	Cell output, 15 patients	
	Minimum	Maximum
Total	1.38×10^6	26.25×10^6
Small lymphocytes*	1.09×10^6	24.33×10^6
Large lymphoid cells and medium, basophilic, lymphoid cells*	2×10^4	77×10^4
Macrophages*	0	19×10^4
Hodgkin's cells†	0	16
Reed-Sternberg cells†	0	0.2

* Based on differential counts of 5,000–15,000 cells.
† Determined from filter specimens.

DISCUSSION

The present studies of peripheral lymph give information about the migration of cells through the tissue from which the lymph is collected and afford the opportunity to evaluate the participation of B-lymphocytes in this migration. We know from studies in sheep that the cell number in peripheral lymph is relatively low, 100–700 cells per mm³ with about 80% lymphocytes and 5–20% monocytes (*11*). Data from man have not been published. Examination of the central lymph gives additional information about the release and migration of cells from the lymph nodes. The central lymph normally contains a high number of cells, most of which are recirculating, small lymphocytes (*1*); monocytes are rare (*3*).

The present study reveals that the cell content of the peripheral lymph from the leg is low in man and about the same as that found in sheep. Most of the cells are small lymphocytes. As in sheep, there is a high percentage of macrophages compared to the percentage of these cells in central lymph and peripheral blood.

In Hodgkin's disease, a clear correlation has been demonstrated between the lymphocyte

TABLE 4.—*Observation of Reed-Sternberg and Hodgkin's cells in the thoracic-duct lymph. Lymph node involvement directly related to thoracic duct*

Case No.	Reed-Sternberg and Hodgkin's cells	Region of involvement			
		Abdominal			
		Pelvic only	Pelvic and para-aortic	Mediastinal	Left supra-clavicular
1	+	−	+	+	+
2	+	−	−	−	+
3	+	−	+	+	−
4	−	−	+*	+	+
5	+	−	+	−	+
6	+	−	+	−	−
7	−	+*	−	−	+
8	+	−	−	+	+
9	+	−	+	−	+
10	+	−	+	+	+
11	+	−	+	−	+
12	−	−	−	−	−
13	+	−	+	+	+
14	−	−	+*	+	−
15	−	−	−	−	−

* Below L IV level.

number in peripheral blood and that in peripheral and central lymph. This might indicate that a certain percentage of blood lymphocytes migrate into the tissues and recirculate normally. In the peripheral lymph, no B-lymphocytes were observed in the Hodgkin's disease patients. This indicates, but does not prove, that the T-lymphocytes are the cells migrating into the tissues. In CLL patients there were some B-cells in peripheral lymph, but few compared to the number of these cells in the blood, with one exception. This shows at least the abnormal B-cells do not migrate to the same extent as the T-cells. The Stage IV Hodgkin's disease patients had remarkably few lymphoid cells in the peripheral lymph, probably reflecting a lymphopenic interstitium. It is an interesting question if this can account for the decreased skin reactivity observed in some Hodgkin's disease patients and the discrepancy observed between in vivo and in vitro response to anigens.

The large lymphoid cells found in central lymph in patients with abdominal lymph-node involvement are probably released from the affected lymph nodes, since not many were found in peripheral or central lymph in the patients without intra-abdominal lymph-node involvement. Most likely, the basophilic lymphoid cells in peripheral blood described by Crowther et al. (*12*) are from the same sources and they express immunologic reactions going on in the nodes involved by the disease.

Monocytes and macrophages were numerous in the thoracic-duct lymph in some of the Hodgkin's disease patients. Also in the peripheral lymph, there was a high percentage of macrophages, which might have been caused by the low lymphocyte count. The interpretation of this finding is therefore uncertain, and further studies of the monocyte and macrophage kinetics are necessary.

The central lymph studies show that Hodgkin's and Reed-Sternberg cells migrate along the lymph channels. There is strong evidence that these cells are malignant (*13*). These cells were found in the thoracic duct in all patients with involvement of the upper abdominal nodes. They could not be demonstrated, however, in 3 patients with involvement of the lower abdominal nodes only. The reason for this might be that the huge Hodgkin's and Reed-Sternberg cells are trapped by apparently normal para-aortic nodes craniad to the involved nodes. This makes it reasonable to assume that lymph nodes regional to an involved lymph node area can be continuously exposed to Hodgkin's and Reed-Sternberg cells. Probably most of these cells are destroyed by immunologic reaction within the nodes. If such immunologic destruction occurs, then lymph nodes regional to an involved area will probably have more specific immunocompetent cells than nodes not exposed to these cells. Possibly, therefore, Hodgkin's and Reed-Sternberg cells are sometimes destroyed in regional nodes but not if they settle in nodes at a certain distance; this could account for the disorderly spread of the disease observed in some cases.

Note added in press: After presentation of this paper a group of Hodgkin's disease patients has been studied 5–23 months after radiotherapy. Peripheral lymph was lymphopenic, while blood had a normal lymphocyte count and contained an average of 50% B-lymphocytes compared to about 10% in untreated Hodgkin's disease patients.

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Consistency of Histologic Subtypes in Hodgkin's Disease in Simultaneous and Sequential Biopsy Specimens [1,2]

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SUMMARY—In patients with Hodgkin's disease, diversity of histologic types in tissues removed simultaneously from different anatomic sites is the exception rather than the rule. More common is the observation of change in histologic type in sequential biopsy material, although a remarkable constancy is evident in nodular sclerosis (92.7%), often over many years of observation. Lymphocyte predominance, in contrast, is a relatively labile form in which the cellular composition of the diagnostic biopsy is maintained in only 38.5% of cases. A change in histologic type in sequential biopsy material, when it occurs, is almost always toward a histologically more malignant form of Hodgkin's disease such as lymphocyte predominance to mixed cellularity, or lymphocyte predominance and mixed cellularity to lymphocyte depletion. The validity of these findings in sequential biopsies was strengthened by the observation of identical histologic subtypes in biopsy specimens removed simultaneously from different anatomic sites. There was not only a striking constancy of histologic type noted between diagnostic biopsy and laparotomy material, but within the nodular sclerosis group, there was also constancy with respect to collagen-band thickness and composition of the cellular nodules.—Natl Cancer Inst Monogr 36: 253–260, 1973.

IN THEIR classical paper on malignant lymphomas, Gall and Mallory (*1*) pointed out that a cytologic classification depends for its validity and applicability on the constancy of cell types over months and years of observation. They stated, with reference to malignant lymphomas in general, that great constancy does in fact prevail, and though variations do occur, they are practically limited to degrees of differentiation without shifts between one major group and another. This statement was made long before the advent of the modern histologic classification of Hodgkin's disease introduced by Lukes and co-workers (*2*) and modified at the Rye conference (*3*).

This presentation reviews our observations on the persistence of histologic subtypes of Hodgkin's disease in sequential biopsies (*4*) and compares them with similar data reported in the literature (*5, 6*) and collected by ourselves.

MATERIALS AND METHODS

The diagnostic lymph-node biopsy sections, as well as the laparotomy material from 39 patients with Hodgkin's disease surgically staged

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[2] Supported in part by Public Health Service training grant CA05183 from the National Cancer Institute.

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[4] We express our gratitude to Robbie Fuqua for help in preparation of the manuscript.

at the University of Chicago Hospitals, were reviewed. In 21 patients who had subdiaphragmatic disease, we attempted to classify histologically *all* involved specimens using the Rye modification of the Lukes and Butler classification. All tissues examined were stained with hematoxylin and eosin. In tissue sections classified as nodular sclerosis (NS), the collagen-band thickness was graded on a 0–4+ scale. Biopsy sections in which the collagen-band thickness was scored as 0 or ± were reported as the cellular phase of NS. In such instances, collagen was either absent or scanty, but lacunar cells were numerous within cellular nodules as described and illustrated previously (*4, 7*). A grading of 4+ indicated very thick collagen bands with only small residual cellular islands. In addition, the composition of the cellular nodules was subclassified as lymphocyte predominance (LP), mixed cellularity (MC), or lymphocyte depletion (LD). The materials and methods used in the study of sequential biopsy material were presented previously (*4*). Briefly, in 94 patients, ≧2 lymph node biopsies were performed during the patient's illness. Thirteen patients who had received chemotherapy before the time of lymph node biopsy, or radiotherapy to areas from which lymph nodes were later biopsied, were considered ineligible for entry into this study. In the remaining 81 patients, each biopsy was histologically classified, and the persistence or change in histologic type was recorded.

RESULTS

Simultaneous Biopsy Specimens (Laparotomy Material)

In the 21 patients with intra-abdominal Hodgkin's disease, histologic classification of the *diagnostic* lymph node biopsy revealed 13 patients with NS, 5 with MC, and 3 with LP. In the latter 3 patients, a nodular architectural pattern was observed as previously described by Rappaport et al. (*8*) and Lukes et al. (*2*). The histologic classification of the tissues obtained at laparotomy was identical to that of the diagnostic biopsy in 20 of 21 patients (table 1). In the one exception, the diagnostic lymph-node biopsy showed NS with a depletion of lymphocytes within the cellular nodules. Review of the tissue obtained at laparotomy revealed Hodgkin's disease with LD in only a portal node and an omental mass. No evidence whatsoever of collagen-band formation or of lacunar cells was noted in these sections. Elastica stains of sections of the portal node revealed vascular invasion.

Table 1.—*Histologic classification of diagnostic biopsy and laparotomy material in 21 patients with subdiaphragmatic involvement*

Case No.	Histologic classification: Diagnostic biopsy	Laparotomy material*
1	NS	NS (S,N)
2	NS	NS (S,N)
3	NS	NS (S,N)
4	NS	NS (S,N,L)
5	NS	NS (N)
6	NS	NS (N)
7	NS	NS (S,N)
8	NS	NS (S,N)
9	NS	NS (S,N)
10	NS	NS (S,N)
11	NS	NS (S,N)
12	NS	NS (N)
13	NS	LD (N)
14	MC	MC (S,N)
15	MC	MC (S,N)
16	MC	MC (N)
17	MC	MC (S,N)
18	MC	MC (N)
19	LP (nodular)	LP (nodular) (N)
20	LP (nodular)	LP (diffuse) (S)
21	LP (nodular)	LP (diffuse) (S)

* N=lymph node, S=spleen, and L=liver.

The remaining 12 patients with NS in both diagnostic lymph-node biopsy and laparotomy material were further studied with respect to thickness of the collagen bands and nature of the cellular composition. In table 2 a remarkable degree of constancy of these histopathologic

TABLE 2.—*Histopathologic features in 12 patients with NS in both diagnostic lymph-node biopsy and laparotomy material**

Biopsy material	Patient No. 1	2	3	4	5	6	7	8	9	10	11	12
Diagnostic Bx	2+/MC	2+/MC	2+/LP	2+/MC	2–3+/LP	2+/LP	±/LP	2–3+/MC	3+/MC	±/MC	2–3+/MC	3–4+/MC
Diagnostic Bx site†	(LS)	(C)	(M)	(RI)	(RS)	(LS)	(C)	(RC)	(LS)	(A)	(RS)	(LI)
Spleen	1+/MC	2+/MC	2+/LP→MC	2+/MC	—	—	±/LP	2–3+/MC	2+/LP	2+/LP	2+/MC	—
Splenic node	2+/MC	1+/MC	1+/LP	U‡		—	—	2+/MC	1+/LP	1+MC	—	—
Portal node	1–2+/MC	—	1+/LP	±/LP	1–2+/LP	—		2+/MC	1+/LP→MC	3+/LP	—	—
L. para-aortic node	1–2+/MC	2–3+/MC	1–2+/LP	U	—	—	±/LP	2+/MC (↑eos)	3–4+/MC	—	2+/MC	—
R. para-aortic node	1–2+/MC		2+/LP	—	—	2+/MC	±/LP	2+/MC (↑eos)	3–4+/MC	2+/MC	—	—
L. iliac node	1+/MC		—		—	—		—	—	—	—	—
R. iliac node	2+/MC		—		—	—		—	—	—	2+/MC	U
Mesenteric node	1–2+/MC		—			—	—	—	—	—	—	—
Liver (wedge)§	—	—	—	2–3+/MC	—	—	—	—	—	—	—	—

* *Code for abbreviations in table*: C/CC: signifies collagen-band thickness/cellular composition of node sections; thickness graded as 0, ±, 1+, 2+, 3+, 4+; —: signifies no evidence of Hodgkin's disease; and blank space: signifies no biopsy performed. L = left, R = right, C = cervical, A = abdominal or retroperitoneal, M = mediastinum, I = inguinal, and S = supraclavicular.

† Bx = biopsy.

‡ U: unclassifiable lesion; i.e., in all of the above cases, this involved a relatively small area of NS in which further subclassification was not possible.

§ All needle biopsies of both lobes negative.

parameters is seen, with the collagen-band thickness and cell composition varying only slightly. A difference in the collagen-band thickness between the diagnostic lymph-node biopsy and laparotomy sections, which represented >1 unit on the 0–4+ grading scale, was seen in 3 patients (#4, 9, and 10) and involved only 6 of a total of 44 laparotomy specimens studied.

The cellular phase of NS was observed in 3 patients (#4, 7, 10). In patient #4 the diagnostic lymph-node biopsy obtained from the right inguinal region presented the characteristic histologic picture of NS, with collagen-band thickness graded as 2+ and a cell composition of MC. The spleen and a wedge biopsy of the liver showed NS with 2 and 2–3+ collagen, respectively, and an MC composition in both. The portal node revealed NS with only ± collagen and LP. The left para-aortic node contained a focus of Hodgkin's disease too small to be subclassified with certainty regarding collagen thickness or cell composition. In patient #7, all biopsy material was classified as the cellular phase of NS with LP. In patient #10, only the diagnostic biopsy specimen, a retroperitoneal lymph node, showed the cellular phase of NS. This patient was surgically staged a few weeks later; the spleen, splenic hilar, and portal and right para-aortic lymph nodes all revealed the characteristic histologic picture of NS.

The cellular composition within the NS group was remarkably constant with only 7 laparotomy specimens differing slightly from the diagnostic node. In patients #4, 6, and 10, the cell composition ranged from LP in some sections to MC in others. In patients #3 and 9, it could not be established with certainty as LP or MC. Details regarding cell composition in all 12 patients are given in table 2.

Sequential Biopsy Material

In the study of sequential biopsy material (*4*), the histologic subtypes of Hodgkin's disease remained constant over long observation periods in most patients. The same histologic picture was observed in 81.9% (50/61) of the patients who had 2 lymph node biopsies and in 75% (12/16) who had ≧3 biopsies. Among histologic subtypes, the highest degree of persistence was observed in Hodgkin's disease of the NS type (51/55 or 92.7%),[5] and the lowest in Hodgkin's disease of the LP type (38.5%). Details relating to the persistence and/or change in histologic types are shown in tables 3–7. Progression, when it occurs, is almost always toward a histologically more malignant form of Hodgkin's disease. In this study, certain pitfalls in the histologic classification of Hodgkin's disease became apparent. First, when a fragment of lymph node tissue is submitted for study, a classification of Hodgkin's disease should not be attempted, though the diagnosis of Hodgkin's disease may be established. A small tissue sample may show a cellular composition consistent with Hodgkin's disease with either LP, MC, or LD, but it is not possible to tell whether one is dealing with one of these histologic types or with NS Hodgkin's disease with a corresponding cellular composition. Second, when a small area of lymph node tissue showing the features of NS is seen, or when the lymph node is partially involved by NS, this material can be classified with certainty as NS Hodgkin's disease. This observation was first made by Hanson (*9*) who studied 251 patients with special reference to NS Hodgkin's disease. He found that the course of the disease and the survival of patients with partial involvement of the lymph node by NS Hodgkin's disease conform to those of patients with complete nodal involvement by this histologic subtype.

TABLE 3.—*Persistence and change in histologic type when the initial biopsy showed LP*

Histologic types	Number of available sequential biopsies 2	≧3
Persistence of LP	4	1
Change to: NS	0	1
MC	2	1
LD	2	1
U*	1	0

* U=unclassifiable—lymphoreticular (L-R) type of Rosenthal. (Rosenthal SR: Significance of tissue lymphocytes in the prognosis of lymphogranulomatosis. Arch Pathol 21:628–646, 1936)

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[5] Including 7 cases in which the diagnostic biopsy sections were classified as the cellular phase of NS.

TABLE 4.—*Persistence and change in histologic type when the initial biopsy showed NS*

Histologic types	Number of available sequential biopsies 2	≥3
Persistence of NS	37	7
Change to: LP	1	0
MC	2	1
LD	0	0

TABLE 5.—*Persistence and change in histologic types when the initial biopsy showed probable NS (cellular phase)*

Histologic types	Number of available sequential biopsies 2 [interval between biopsies] (yr)		≥3 [interval between biopsies] (yr)	
Persistence of probable NS [cellular phase]	1	[8.3]	1	[0.1, 4.4]
Change to: LP	0		0	
NS	3	[5.5] [1.8] [3.9]	2	[2.3, 0.5, 1.5] [2.8, 1.4]
MC	0		0	
LD	0		0	

TABLE 6.—*Persistence and change in histologic type when the initial biopsy showed Hodgkin's disease with MC*

Histologic types	Number of available sequential biopsies 2	3
Persistence of MC	4	1
Change to: LP	0	0
NS	0	0
LD	2	0

TABLE 7.—*Persistence and change in histologic type when the initial biopsy showed Hodgkin's disease with LD*

Histologic types	Number of available sequential biopsies 2
Persistence of LD	1
Change to: LP	0
NS	0
MC	1

DISCUSSION

In patients with Hodgkin's disease, diversity of histologic subtypes in tissues removed simultaneously from different anatomic sites is the exception rather than the rule. Rarely, different areas of the same biopsy section may show different histologic types (*10*). More common is the observation of change in histologic type in material from sequential biopsies (table 8), although a remarkable constancy is evident in NS (92.7%), even over many years of observation (table 9). This percentage is for all practical purposes close to the maximum accuracy that can be achieved in the histologic classification of Hodgkin's disease (*6, 11, 12*) (table 10). In contrast, LP is a labile form in which the cellular composition of the diagnostic biopsy is maintained in only 38.5% of patients. "Transitions," therefore, are seen almost exclusively between histologic types of Hodgkin's disease other than NS, such as from LP to MC or LD, and from MC to LD.

The validity of our observations in sequential biopsies depends on the assumption that at a given time there is either great similarity or identity of histologic subtypes in different anatomic sites. That this is true was established by Kadin et al. (*5*) and Dorman (*6*), in studies of patients with Hodgkin's disease staged by

TABLE 8.—*Persistence and progression of histologic types in sequential biopsies from 81 patients with Hodgkin's disease**

Histologic classification of biopsy sections						
	Subsequent biopsies					
Initial biopsy	LP	NS	MC	LD	U	Persistence (%)
LP	5	1	3	3	1	38.5 [5/13]
NS†	1	51	3	0	0	92.7 [51/55]
MC	0	0	5	2	0	71.3 [5/7]
LD	0	0	1	1	0	[1/2]
U	0	1	1	1	1	[1/4]

* Cases *above diagonal* represent a change in histologic type to a histologically more malignant form; those *below diagonal* represent the reverse progression; those *within diagonal* represent persistence of subtype.

† Includes 7 cases in which the biopsy sections were classified as the cellular phase of NS.

Table 9.—*Persistence and progression of histologic types of Hodgkin's disease in sequential biopsies in relation to time interval between biopsies*

Initial histologic classification	Number of cases	Interval between biopsies*	Aerage interval (yr)
LP	13[16.9%]	0.8–11.6	3.2
NS [cellular phase]	48 [71.4%]	0.1–17.3	2.9
NS	7	0.1– 8.3	3.0
MC	7[9.1%]	0.1– 1.6	0.8

* Expressed as range in years.

Table 10.—*Observer agreement on the classification of Hodgkin's disease*

Reference (yr) and observers	Frequency of concurrence (%)	
	2 observers	3 observers
Keller et al. [(*11*), 1968] (Keller, Lukes, and Rappaport)	92.0	67.0
Coppleson et al. [(12), 1970] (Strum, Graff, and Rappaport)	91.3	54.2
Dorfman [(6), 1970] (Berard, Dorfman, and Lukes)	94.0	89.0

laparotomy. They were the first to describe a striking consistency between the histologic types noted in the diagnostic lymph-node biopsy and that of the involved abdominal lymph nodes and spleen. Studies at the University of Chicago Hospitals and Clinics and at Brooke General Hospital, Fort Sam Houston, Texas, confirmed their findings and showed a constancy not only in histologic type but also in collagen-band thickness and cellular composition within the NS class. These findings therefore differ from those of Razis et al. (*13*) who stated that 57 of a total of 1,102 cases of Hodgkin's disease showed different histologic types in the same biopsy section. Their study, however, was performed before the introduction of the Lukes and Butler classification. Recently, Smithers (*14*) stated that progression from one histologic type to another is commonly seen and that nodes showing more than one histologic type can be found at the same time in the same patients. Farrer-Brown et al. (*15*) reported differences in histologic type between the original biopsy and spleen in 55% of their patients subjected to surgical staging. In 6 patients, Hodgkin's disease with LD was seen in the spleen sections, whereas the diagnostic biopsy showed either LP, NS, or MC. In 5 patients, the spleen sections showed LP, whereas the diagnostic biopsy was classified as NS or MC. In contrast, the abdominal lymph nodes and diagnostic biopsy were of identical histologic type in 10 of 13 patients.

In patient #10 of the present study, the only exception to the general observation of constancy of subtype in simultaneous tissue examination, the diagnostic lymph-node biopsy was classified as NS with LD within the cellular nodules, whereas a portal node and an omental mass showed Hodgkin's disease with LD. Vascular invasion was prevalent in the portal node. This may reflect the known tendency for vascular invasion to be associated with: 1) Hodgkin's disease of the LD type, 2) the number of mononuclear cells with a nuclear structure similar to that of Sternberg-Reed cells seen in the lymph node sections independent of histologic type, and 3) an increased degree of aggressiveness in which a high frequency of extranodal involvement is seen (*16–18*). Conceivably in patient #10, an increased degree of aggressiveness was associated with vascular dissemination of clusters of Sternberg-Reed cells and their mononuclear variants, without either collagen-band formation or the presence of lacunar cells. Tissue sections of the diagnostic biopsy were examined for vascular invasion, which, however, was not observed. This patient developed a pleural effusion 2 weeks after laparotomy which cytologically was consistent with Hodgkin's disease. He died 3 weeks later.

In 2 of the 3 patients in which the diagnostic biopsy showed LP (nodular), laparotomy specimens showed LP (diffuse). This agrees with the findings of Rappaport et al. (*8*) who in 1956 noted that the nodular form of Hodgkin's paragranuloma may or may not be maintained in follow-up biopsies, but that the cytologic picture remained consistent.

The thickness of the collagen bands, within the NS group, may have greater clinical significance than is apparent from this study. First, the cellular phase of NS, when observed in a peripheral diagnostic biopsy, appears to be associated with twice as high a frequency of abdominal disease as compared to classic NS with broad bands of collagen (*5*). The cellular phase also appears more frequently in children in the first decade of life (*7*), and subjectively such patients appear to have more extensive disease. Second, patterns of spread of disease may become clearer if the collagen-band thickness reflects the duration of the disease process within a particular anatomic site. In the present study, conclusions of this nature could not be made because of the few cases and, more importantly, because of a lack of consistent sampling of nodes in such sites as the celiac axis and high para-aortic regions at the level of the twelfth thoracic and first and second lumbar vertebrae.

It is emphasized that all sequential biopsies were obtained from previously untreated areas because of the inadvisability of classifying lymph node sections from patients with Hodgkin's disease previously treated with either systemic therapy or radiation therapy. When residual lymph-node disease is detected following therapy with either modality, lymphocytes, plasma cells, and eosinophils may be preferentially depleted. The apparently more treatment-resistant residual cells, predominantly the Sternberg-Reed cells and the mononuclear cells with similar nuclear structure, may give such nodes a histologic appearance of Hodgkin's disease with LD. Radiation therapy may result in a lymph node with the appearance of obliterative fibrosis when eradication of disease is complete; but when eradication of disease is incomplete, it may result in cellular changes similar to those described above except for the additional finding of an increased amount of fibrosis. In both situations, a histologic classification of Hodgkin's disease with LD would be in error unless the pretreatment biopsy showed this histologic type. Such treatment may significantly alter the cellular composition and thus the classification of Hodgkin's disease with LP and MC, while in NS the architectural pattern is basically preserved, possibly allowing for an accurate histologic classification despite therapy. In some cases of NS, however, radiation treatment may increase the amounts of fibrous tissue simulating the appearance of the obliterative phase of NS. This histologic picture is frequently confused with Hodgkin's disease with LD, in which diffuse fibrosis is prominent. Histologic classification of treated lymph nodes should therefore be avoided unless the architectural pattern of NS is recognized.

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Invited Discussion: Synthesis and Implications for the Etiology, Pathogenesis, and Spread of Hodgkin's Disease[1]

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I WILL TAKE an iconoclastic view of some of the foregoing presentations and discussions. We have all agreed for decades that Hodgkin's disease is very peculiar. There has been great debate about whether it is a reactive process, a neoplasm, or a combination of the two. We will continue to argue about that until we know the fundamental nature of the disease and something about the significance of "the host response" to the disease. In the last day and a half we have seen repeatedly data indicating that the cells we refer to as Hodgkin's cells and Reed-Sternberg cells are, in terms of their kinetics and often their morphology, so inextricably linked to the cells we interpret as reactive, that it is only by classic morphologic dogma that pathologists consistently call one class neoplastic and the other class reactive. I was very interested in the presentations in the epidemiology session, and particularly in Wright's comment (*1*) that, in Uganda, he could detect no apparent geographic variation in the incidence of Hodgkin's disease like the patterns seen for malaria and Burkitt's lymphoma. To me this implies there may be a factor involved in Hodgkin's disease which is more related to host susceptibility than to environment.

Let me present a hypothesis, which may perhaps not be new, but which has not been presented in a straightforward way at this Symposium. Is it conceivable that Hodgkin's disease is not a disease with neoplastic cells to which there is a vigorous host response, but that Hodgkin's disease is itself a malignant cellular immune process in which all of the cells are involved? Could this relate, for example, to the bimodal age distribution of Hodgkin's disease? If host susceptibility is a factor, is there anything in the bimodal age-specific incidence of Hodgkin's disease which might make sense in that context?

Is it possible that the early peak relates to thymic involution? If there is a long latency period for the development of Hodgkin's disease as for many other neoplasms, then perhaps the disease we see from puberty through the 35-year-old age group represents the end result of some factor which is creating an unstable situation within the immune system and which may increase the susceptibility to neoplastic transformation. The older age peak might relate to other diseases we think are related to the immune system such as multiple myeloma, which characteristically occurs with an age peak like that of Hodgkin's disease in the older age group. Perhaps this disease has its peak incidence at times when the immune system is undergoing changes in balance.

The agents which might cause Hodgkin's disease have been discussed—Epstein-Barr virus (EBV) particularly—and if this virus, or an agent similar to it is responsible, it certainly causes no apparent disease in most people. If EBV is involved, and it seems to be very strongly related to infectious mononucleosis, there are two features of infectious mononucleosis that have not been pointed out. If you look at the bone marrow of patients with infectious mononucleosis you may find atypical lymphocytes similar to those you see in the peripheral blood, but you may also see hyperplasia of other marrow elements, sometimes a conspicuous granulocytic hyperplasia not unlike that one occasionally sees in Hodgkin's disease. Dorfman (*2*) commented on the epithelioid granulomas seen in Hodgkin's disease. Epithelioid granulomas in the bone marrow of patients with infectious mononucleosis are also well known.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

If Hodgkin's disease is a neoplasm, if all the cells are involved, how can we understand the histologic parameters we call lymphocytic predominance, mixed cellularity, lymphocytic depletion, and so on? Strum, Park, and Rappaport (*3*) have pointed out that cells morphologically indistinguishable from Reed-Sternberg cells can be seen in several other reactive or neoplastic situations. In other neoplastic situations, pathologists interpret such cases as not being Hodgkin's disease by referring to the background cell population with which these binucleate cells are associated. We also use the differentiation of the participating elements we see to help us identify other neoplasms. In Hodgkin's disease, when cells are interpreted as Reed-Sternberg cells, why not consider the differentiated cells with which they are associated as a possible clue to their nature, rather than as a response of the host? Could it be that the transformation from lymphocytic predominance to mixed cellularity to lymphocytic depletion is, in fact, a progressive dedifferentiation of the tumor, such as we see in some other neoplasms? Are the polymorphonuclear leukocytes and benign-appearing macrophages often associated with them related to some of the chemotactic factors which we have been told exist as part of the normal cellular immune reaction?

Kaplan's group (*4, 5*) showed data indicating that perhaps a defect in the cellular immune system occurs very early in Hodgkin's disease. If the cellular immune system is disturbed in Hodgkin's disease, perhaps it is disturbed in a way in which its better differentiated forms can mimic many of the inflammatory components of a normal cellular immune reaction, but which, as it progresses and as the cells comprising it become more and more abnormal, loses its ability to function, and reverts then to a tumor in which slowly the normal lymphoid elements, the inflammatory elements, drop out. Thus we end up with only a proliferation of undifferentiated cells that are no longer morphologically suggestive of the nature of the disease from which they arose. It is often a problem for the pathologist confronted with such a disease to differentiate Hodgkin's sarcoma from other malignant tumors. Sometimes we can go back and look at earlier biopsies from the same patient and then we say, "Oh, this is Hodgkin's disease." We do it by looking at the cellular environment.

Good (*6*) pointed out that there are 2 immune systems and that multiple myeloma and agammaglobulinemia may be related to the B-cell system. Hodgkin's disease patients seem to have a defect most consistent with T-cell problems. It is interesting that in the further discussion of that phenomenon Good stated that, in patients with known congenital immune-deficiency states, Hodgkin's disease does not seem to be increased in frequency. I have reviewed a number of patients at the National Institutes of Health with ataxia-telangiectasia and Wiskott-Aldrich syndrome; I have never seen a convincing example of Hodgkin's disease in this population either. Could it be that Hodgkin's disease is not a disease which affects the immunologically weak, but a disease that affects the immunologically competent?

Bodmer (*7*) showed some trends in HL-A types which he said may be related to an unknown disease-susceptibility factor — perhaps linked to an HL-A factor. I was very interested in McDevitt's comment (*8*), if I understood him, that in the mouse, immune-response (*Ir*) genes are dominant. This might be interpreted to indicate that in Hodgkin's disease *Ir* genes are not involved because they would confer protection. There is, however, an alternative interpretation. *Ir* genes could be involved if it is the patient who is a "superimmune" responder who is most susceptible to Hodgkin's disease. The same kind of challenge which might in another individual cause no apparent disease or only infectious mononucleosis might, in the uniquely susceptible person with a hypersensitive immune-response profile, trigger a process such as Hodgkin's disease.

I was interested in hearing from Order et al. (*9*) that in 7 of 14 histologically negative spleens of patients with Hodgkin's disease he could identify Hodgkin's associated antigens. I would hope that his future studies on peripheral blood and "reactive" nodes in Hodgkin's disease may shed light on the potential significance of this tumor associated antigen.

Miller (*10*) showed some of the background states associated with leukemia or lymphoma. You will remember that the chromosomal anomaly group was most closely linked to leukemia, whereas a series of other factors seemed to be related to lymphoma. Among that latter group, one chemical agent, Dilantin, was listed. To my knowledge, Dilantin is the only agent which creates in a significant number of people a Hodgkin's-like lesion. It is interesting that Dilantin is also an agent which is able to elicit a cellular immune response and which at the same time may be a mild immunosuppressant. It may in susceptible individuals create a situation in which the host is stimulated to respond but is responding with a labile immune system. Perhaps this relates to my earlier comments about the bimodal age peak of Hodgkin's disease and the possible instability of the immune system at two different periods in our lifetime.

With regard to the spread of Hodgkin's disease, I do not think we yet know whether cells spread or agents spread or influences spread. Certainly, vascular invasion, as Strum et al. (*11–13*) have shown, may occur in a certain proportion of instances, usually in advanced Hodgkin's disease. But there is something very peculiar about the spread of Hodgkin's disease. If Hodgkin's disease affects a susceptible population of people and also, as Smithers (*14*) has suggested, affects a susceptible population of the lymph nodes in the body, namely, a very high propensity to involve the cervical nodes, it is equally intriguing that it has a very marked propensity to involve a specific region within the node. I can illustrate this with a node containing early Hodgkin's disease in which the outer cortical regions and the germinal center apparatus are intact and the paracortical regions are where the Hodgkin's disease is localized. This is a consistent and invariable finding in cases with partial involvement of lymph nodes by Hodgkin's disease. It is interesting and relevant to this discussion that the paracortical region of the node is also the "thymus-dependent" region to which the T-lymphocytes migrate.

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SUMMARY OF INFORMAL DISCUSSION ON HISTOPATHOLOGY AND EXPERIMENTAL CYTOLOGY

H. S. Kaplan offered comments which he hoped would stimulate discussion in the Workshops. At the risk of exciting a little hostility in some of his good friends who are pathologists, he urged them to break the shackles of morphology, if possible. It may be asking a lot for a pathologist to liberate himself from morphology, since this is the be-all and end-all of his existence. Nonetheless, he had been struck, especially in the tissue culture studies, with the tremendous limitations which we suffer in our inability to identify these cells except in morphologic terms, and with the fact that those terms simply are not good enough. Without singling out any one individual, if one wishes to say that a given cell in a tissue culture is a lacunar cell, the conviction must be in the eye of the beholder because nothing but morphology supports that view.

He was also impressed with the fact that we have not come very far with tissue culture studies. If one goes back to the older literature, one can readily find in papers by Mankin, Meyer, and Lewis, as far back as the 1930's, and in papers by Hoster, Rottino, and others, around 1950, clear descriptions of some of the same cells that we are shown today in cell culture and that were just as unidentifiable then as they are today. He pointed out that the immunologists, as **Doctor Good** had brought out, had encountered experimental evidence separating populations of lymphocytes into 2 classes which they call T- and B-cells. This was initially based on certain functional responses. They have now gone on to develop serologic and other methods to distinguish the T- and the B-cells from each other. How long would the immunologists have had to wait if they had depended on morphology to distinguish 2 morphologically identical populations of lymphocytes? He believed that the time had come when the pathologists must be aggravated, provoked, stimulated, encouraged, and harassed into using all of the modern techniques possible: chromosome counts, tritiated thymidine uptake, specific serology, and any other methods which are not strictly morphologic, in the hope that we can progress beyond our present position.

H. Rappaport replied that the purpose of the pathologist is to do the best he can with the tools at his disposal. What we have done is to stimulate the molecular biologist, the immunologist, and the cytogeneticist to do precisely what Doctor Kaplan is asking us to do. We cannot do it by ourselves.

SESSION 3

DIAGNOSTIC EVALUATION AND CLINICAL STAGING

Chairman: Saul A. Rosenberg

INTRODUCTION

This session marks a transition to more clinical matters. The first one is devoted to "Diagnostic Evaluation and Clinical Staging," which has been the subject of intensive clinical studies over the past decade. A half day's session cannot do justice to all that has been done or to more than a few of the questions that have been raised. However, in April of last year there was an entire symposium dedicated to this subject (now published in *Cancer Research,* 31:1707–1870, 1971). In the audience are many members of the group that participated in that Ann Arbor symposium. We have therefore reduced the program relating to evaluation and staging, choosing just to select certain questions that were raised at the last meeting, rather than giving a comprehensive review of this problem.

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Roentgenologic Aspects of Hodgkin's Disease.
I. Current Role of Lymphangiography [1,2]

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SUMMARY—Lymphangiography demonstrated a 98% (105/107) accuracy in prediction of histologically normal or abnormal lymph nodes. There were no false negatives for Hodgkin's disease in 69 consecutive, untreated patients. However, due to a high incidence of benign histologic abnormalities, a 26% false-positive error occurred when prediction of involvement with tumor was attempted. In 15% of all patients that had a relapse, radiographic detection of changes in opacified retroperitoneal lymph nodes was the first manifestation of initial relapse. Our experience with repeat lymphangiography indicated that in approximately 30% a previously normal lymphangiogram changes to an abnormal lymphangiogram. In almost 10%, the repeat study showed distinct changes in the opacified lymph nodes—not due to recurrent tumor. Lymphangiographically opacified lymph nodes serve as important guides to surgical sampling and in radiotherapy field planning.—Natl Cancer Inst Monogr 36: 271–276, 1973.

AT STANFORD University School of Medicine, we now study all patients with previously untreated Hodgkin's disease by means of stereoscopic frontal and lateral chest radiographs, supplemented by full lung tomography in the frontal projection. In contrast to others (*1*), we do not frequently gain new information from frontal tomography that would change either stage or treatment. However, we found lateral tomography helpful in selected instances, not only in evaluating equivocal bronchopulmonary (hilar) adenopathy but also in demonstrating pulmonary parenchymal lesions obscured on frontal views by large, overlying anterior mediastinal masses (*2*).

Lymphangiography is utilized, except in rare instances, where there is a medical contraindication. Inferior venacavography, once performed almost routinely at Stanford, is now rarely used in the initial workup of the patient with Hodgkin's disease. The axial skeleton is radiographically surveyed, and other radiologic procedures are performed as clinically indicated.

These radiographic studies are critical in the initial evaluation of the patient and also serve as sets of baseline data that are important in the detection of relapses. The advent of staging laparotomies prompted some to suggest that lymphangiography is not a necessary part of the initial evaluation (*3*). We refute this concept, not only because the lymphangiogram is important as a guide to surgical sampling and in radiotherapy field planning but also because, in the post-treatment follow-up period, changes in residually opacified retroperitoneal lymph nodes are an irreplacable factor in the diagnosis of relapse (*4, 5*).

We will therefore confine ourselves to presenting what we consider the current role of lymphangiography in patients with Hodgkin's disease.

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ACCURACY

The staging laparotomy permits correlation of the lymphangiographic and histologic findings in resected lymph nodes, thus providing data to determine the accuracy of lymphangiographic diagnoses. Prediction of accuracy based on findings in selected patients might produce spurious results, e.g., when an equivocal lymphangiographic diagnosis is the major indication for laparotomy. The accuracy of the lymphangiogram would be better evaluated from data based on a series of consecutive, previously untreated patients who undergo laparotomy shortly after lymphangiography.

Conclusions are best drawn after careful determination, with the use of postoperative abdominal films, that the lymph nodes removed correspond to those suspect on the lymphangiogram. Simple correlation of the radiographic report with the surgical pathologist's findings will not uncover those instances in which the nodes of radiographic concern were not biopsied. Since foot lymphangiography does not opacify nodes in the splenic hilus, porta hepatis, or mesentery, we think it inappropriate to consider cases as false negative when these nodes are shown to be diseased, but the sampled para-aortic and iliac lymph nodes are lymphangiographically and histologically normal.

Some lymphangiograms are not clearly positive or negative. Many radiologists use the term "equivocal" to encompass this group. We endorse this concept, but have substituted the categories "probably normal" or "probably abnormal" to impart a bias in this uncertain group. If we think a lymph node(s) is abnormal, but due to a benign rather than a malignant disease process (e.g., fatty replacement, hyperplasia, fibrosis, etc.), we qualify the lymphangiographic diagnosis as being "abnormal, due to benign change."

In a continuing prospective study, we correlated the staging lymphangiogram and the postoperative abdominal film with the surgical-pathologic findings. We now have a prospective series of 128 consecutive patients with previously untreated Hodgkin's disease admitted to the protocol since January 1970 (table 1). From this group, 21 patients were excluded for the following reasons: in 3, change of diagnosis from Hodgkin's disease to non-Hodgkin's lymphoma; in 11, no opacified nodes were biopsied, either because of lack of performance of a lymphangiogram, of the laparotomy, or both; and in 7, because the lymphangiographically suspicious or abnormal lymph nodes were not biopsied.

Table 1.—*Radiologic-histologic correlation of lymphangiography on 128 patients with Hodgkin's disease admitted to protocol (Jan. 1, 1970–March 1972)**

Histologic diagnoses	107 Lymphangiogram diagnoses		
	Normal—65	Benign changes—4	Hodgkin's disease—38
Normal—67	65	0	2
Benign changes—12	0	4	8
Hodgkin's disease—28	0	0	28 (74%)

* Exclusions:	
change in diagnosis	3
no node biopsied	11
sampling discrepancy	7
	21

Of the remaining 107 patients, 65 had lymphangiograms interpreted as normal. All biopsied retroperitoneal lymph nodes were histologically normal in these patients. In 4, a lymphangiographic diagnosis of "abnormal, due to benign change" was histologically confirmed. Thus, all 69 lymphangiograms interpreted as being negative for Hodgkin's disease were histologically confirmed. The price for having no false negatives is paid by an increase in false positives. Thus, of 38 patients diagnosed as having abnormal lymphangiograms consistent with Hodgkin's disease, only 28, or 74%, had Hodgkin's disease in the excised lymph nodes. In the remaining 10 patients, only 2 had histologically normal nodes, while the other 8 had histologic abnormalities which were benign, i.e., fibrosis, fatty infiltration, sinus hyperplasia, without evidence of Hodgkin's disease. Thus, although the lymphangiogram interpreted as abnormal due to Hodgkin's disease was very accurate in locating histologically abnormal lymph nodes, i.e., in 36 of 38 patients (95%), the abnormalities in 1 of 5 were benign—and in only 4 of 5 were they due to Hodgkin's disease.

DETECTION OF RELAPSE

Lymphangiography is important in detection of relapses. We support this statement by our results of a recent review of the methods of detection of initial relapse, from 1955 to 1970, in patients with Hodgkin's disease who were treated with a curative intent (*4*). Of 442 patients, 130 had one or more relapses. The methods of detection of the initial relapse in these 130 patients were determined and placed into 1 of 3 categories (table 2).

TABLE 2.—*Method of detection of initial relapse in patients with Hodgkin's disease treated with curative intent (1955–70)*

442 Patients ⟶ 130 patients relapsed (29%)

Clinical alone	46	(35%)
Clinical and radiographic	39	(30%)
Radiographic alone	45	(35%)
	130	(100%)

↓

a) Relapses detected only by radiographically abnormal retroperitoneal lymph nodes	15%
b) Relapses detected only in pulmonary parenchyma and/or mediastinal nodes	14%
c) Miscellaneous (simultaneous abnormalities on chest and abdomen, or bone lesion)	6%
	35%

Slightly over one-third had their relapse detected solely by clinical information, such as provided by physical examination or symptomatology. In an equal number of patients, relapses were detected solely by radiographic findings made on routine surveillance chest and abdominal films in patients who were otherwise asymptomatic and clinically free of disease. In slightly less than one-third, the detection of relapse was made simultaneously by clinical and radiographic means.

Of all 442 patients at risk for relapse after initial therapy, 10% had a diagnosis of relapse initiated from findings on routine radiographs.

Furthermore, of all 130 patients who had relapse, 15% had their relapse detected only by radiographic changes in their retroperitoneal lymph nodes. Most of these relapses were detected by changes in previously opacified nodes—but in 20% the abnormal nodes were appreciated on reopacification lymphangiography performed only because of insufficient residual contrast material from prior study.

REPEAT LYMPHANGIOGRAPHY

To determine the value of the repeat lymphangiogram, we reviewed the initial and second lymphangiograms performed on patients with Hodgkin's disease, ending in December 1971 (*6*). Ninety-nine such patients were found, and the results were classified according to their prior radiotherapy (table 3).

The largest group, 64 patients, had no prior radiotherapy below the diaphragm. Thirteen patients had radiotherapy only to paralumbar lymph nodes, with sparing of the iliac lymph nodes, and the remaining twenty-two patients had radiotherapy to both paralumbar and iliac lymph nodes.

Approximately half of all patients had the first and second lymphangiogram interpreted as normal. Most of these repeat lymphangiograms were performed solely for routine reopacification, but approximately one-third were because of clinical suspicion of relapsing disease.

TABLE 3.—*Repeat lymphangiography in 99 patients with Hodgkin's disease (ending Dec. 1971)**

Treatment group	Neg→neg	neg→pos	neg→RH	or pos→neg pos→pos
No prior radiotherapy to para-aortic or pelvic nodes	37/64	18/64	6/64	3/64
Prior radiotherapy to para-aortic nodes—not to pelvic nodes	7/13	4/13	2/13	0/13
Prior radiotherapy to para-aortic and pelvic nodes	5/22	6/22	0/22	11/22
Total	49/99	28/99	8/99	14/99

* neg=normal lymphangiogram; pos=abnormal lymphangiogram (due to Hodgkin's disease); and RH=lymphangiographic appearance of "reactive hyperplasia" (*see* text).

In almost 30%, the first lymphangiogram was normal, but the second demonstrated evidence of tumor. Of these 28 patients, 14 had retroperitoneal lymph-node biopsies, and in all 14 the radiographic diagnosis was confirmed. In 7 of these 28 patients, the second lymphangiogram was performed solely for reopacification or because of suspicious but not diagnostic changes on surveillance abdominal films. The remainder were repeated for clinical reasons. In this group, the repeat studies at times strengthened the diagnosis of relapse, as suggested by vague symptomatology or equivocal physical findings.

An interesting finding in almost 10% of the patients was the change from a normal lymphangiogram to one where the lymph nodes were larger and bulkier and had a granular, reticulated architecture. These changes represent an interesting, unexplained phenomenon that we loosely termed "reactive hyperplasia," since in 2 of these 8 patients this finding was noted on histologic section. The remaining 6 patients, followed for ≧1 year without intervening therapy, had no evidence of tumor. This further supports the benign nature of this change. When present, these changes occurred diffusely in all opacified lymph nodes, except those previously irradiated.

TABLE 4.—*Comparison of accuracy of lymphangiograhy in Hodgkin's disease**

Investigators	Unselected cases	Previously untreated	Radio-graphic correlation	Total cases	Total after exclusions	Biopsy— / Lag— Number of cases	Biopsy— / Lag— %	Biopsy+ / Lag+ Number of cases	Biopsy+ / Lag+ %	Equivocal Diagnosis Number of cases (%)	Equivocal Diagnosis Biopsy—	Equivocal Diagnosis Biopsy+
Davidson et al. (1968)	No	?	No	31	31	3/4	75	25/25	100	2(6)	0	2
Glatstein et al. (1969)	No	No	No	65	57	14/17	82	23/28	82	13(23)	10	3
Enright et al. (1970)	Yes	Yes	No	68	?66	17/24	71	14/20	70	22(33)	14	8
Lowenbraun et al. (1970)	No	No	No	12	8	2/2		3/6		0		
Rosenberg et al. (1970)	Yes	Yes	No	100	100	55/57	97	17/23	74	20(20)	17	3
Jelliffe et al. (1970)	?	?	No	22	21	10/11	91	6/6	100	4(19)	2	2
Glatstein et al. (1970)	Yes	Yes	No	50	50	17/17	100	14/17	82	16(32)	13	3
Ultmann et al. (1970)	Yes	?	Yes	29	29	13/13	100	6/12	50	4(14)	3	1
Hanks et al. (1971)	No	?	No	14	11	5/6		5/5		0		
Hass et al. (1971)	No	No	Yes	50	43	22/27	100	15/16	94	0		
Pronitz et al. (1972)	Yes	Yes	No	40	36	10/14	71	13/13	100	9(25)	7	2
Zarembok et al. (1972)	Yes	Yes	Yes	28	26	12/12	100	7/11	63	3(12)	3	0
Castellino et al. (1972)	Yes	Yes	Yes	114	107	69/69	100	28/38	74	0		

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* The periods covered in the analysis of Stanford patients are: Glatstein (1969) 1960–Nov. 1968; Enright (1970) July 1968–May 1969; Rosenberg (1970) Aug. 1968–May 1970; Gatlstein (1970) Aug. 1968–April 1969; Castellino (1972) Jan. 1970–March 1972.

DISCUSSION

Various investigators have discussed the accuracy of lymphangiography in diagnosing Hodgkin's disease by use of correlative histologic material from staging laparotomies (table 4) (*1, 7–17*). It is, however, difficult to compare our results to some of these series due to differences in material. Some investigators included previously treated patients or selected, nonconsecutive cases; some did not correlate radiographically the specific nodes biopsied with the pathology report; some considered lymphangiograms as false negative from those cases with histologically normal, opacified nodes but with involved nodes in nonopacified regions. Lastly, in other series, from 6 to 33% of all cases are diagnosed as "equivocal," a category which cannot be analyzed for diagnostic accuracy.

When one considers the larger series of similar composition to ours, a normal lymphangiogram apparently is extremely accurate (approaching 100%) in diagnosis of normal lymph nodes. Although the incidence of false negatives is very small, one must remember that this correlation does not exclude errors due to tumor in nonopacified nodes outside the paralumbar and iliac chains or sampling limitations at the time of laparotomy.

The high incidence (26%) of false positives in our material reflects the surprisingly high incidence of nonmalignant histologic abnormalities in these patients. The detailed pathologic findings will be presented elsewhere. Whether similar abnormalities account for the false-positive rates from other investigators is unknown. Hopefully, this false-positive rate can be reduced by improvement in our ability to recognize benign changes as a separate lymphangiographic entity.

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Whole-Body Radiogallium Scanning for Staging of Hodgkin's Disease and Other Lymphomas [1,2]

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SUMMARY—Total-body scanning with radiogallium (^{67}Ga)-citrate is a useful technique in staging patients with Hodgkin's disease and histiocytic-type malignant lymphoma. Experience with 30 patients indicates an overall accuracy of approximately 80%. Although identification of disease in the abdomen, including spleen, liver, para-aortic nodes, and para-iliac nodes, is difficult due to interference from ^{67}Ga uptake in the liver, colon, and marrow, identification of disease in lymphatic tissues above the diaphragm is highly accurate. The ^{67}Ga scan is particularly useful in identifying unsuspected sites of extranodal involvement. In 3 patients, the ^{67}Ga scan was *the* significant diagnostic study.—Natl Cancer Inst Monogr 36: 277–285, 1973.

IN 1969, Edwards and Hayes demonstrated the usefulness of gallium-67 (^{67}Ga)-citrate as a tumor-scanning agent (*1*). Subsequently, it was reported that this tracer localizes in Hodgkin's disease as well as in the other malignant lymphoreticular neoplasms (*2*). It also localizes in inflammatory tissue and in certain normal organs and therefore cannot be considered specific for neoplastic tissues (*3*). Nevertheless, when a histologic diagnosis of Hodgkin's disease has been established, total-body scanning with ^{67}Ga-citrate can be extremely valuable in determining the extent of disease. Our use of this nuclide in the staging of Hodgkin's disease and other malignant lymphomas was previously reported (*4, 5*). The present communication describes our scanning technique, reviews our results with special emphasis on certain problem areas, and suggests some future modifications in both technique and interpretations which may extend the use of ^{67}Ga scanning in the staging of Hodgkin's disease.

The exact mechanism whereby ^{67}Ga-citrate localizes in tumor tissue is unknown. After intravenous injection, the tracer is rapidly bound to plasma proteins (*6*). ^{67}Ga-citrate disappears from plasma with a biological $T_{1/2}$ of approximately 12 hours (*7*). ^{67}Ga is identified in the lysosomal fraction of tumor ultracentrifugates (*8*) and is believed to localize in a lysosome or lysosome-like organelle (*9, 10*). The relative abundance of lysosomal elements in macrophages and other reticuloendothelial cells may explain the affinity of the nuclide for lymphomatous tissue, for other tumors, and for sites of inflammation. ^{67}Ga localization in tumor may also be promoted in part by leakage of the ^{67}Ga-labeled plasma proteins into tumor sites due to increased protein permeability of the tumor vessels.

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[2] Supported by the American Cancer Society, Inc., The Junior Auxiliary of The University of Chicago Cancer Research Foundation and Goldblatt Brothers Employees Nathan Goldblatt Cancer Research Fund, and the Orin Winters Fund.

[3] Scholar in Radiological Research of the James Picker Foundation.

TECHNIQUE

Carrier-free ^{67}Ga-citrate is used as the scanning agent. It is prepared by deuteron (8 Mev) bombardment of natural zinc [the significant reaction being ^{66}Zn (d,n)^{67}Ga] on the Argonne Cancer Research Hospital's compact cyclotron (Cyclotron Corporation CS–15). Production by this method results in no significant contamination by other long-lived radionuclides (*11*). A 3-mCi dose of the nuclide is administered to the patient by intravenous injection. The total-body radiation dose from ^{67}Ga-citrate is estimated at 340 millirads per mCi (*1*). The initial scan is performed 72 hours after injection. On the evening before scanning, the patient receives 2 ounces of castor oil to evacuate any tracer contained in the feces.

The scan is performed with a dual 5-inch rectilinear scanner using a focused collimator with 9-cm focal length (Ohio Nuclear 38 H). The lower probe scans just below the table top, and the upper probe is located 2–4 cm above the patient. The patient is scanned in the supine position from the top of the head to below the knees. A five-to-one "minification" technique is used, which allows the entire study to be recorded on a 14×17 inch X-ray film.

The major photon emissions of ^{67}Ga and their intensities are as follows: 93 kev (40%), 184 kev (24%), 296 kev (22%), and 388 kev (7%) (*12*). To obtain maximum efficiency, the scanner has been modified with 3 independent pulse-height analyzers for each probe. This permits simultaneous scanning of the 94 kev, 183 kev, and 296 kev peaks of ^{67}Ga and allows the whole-body scan to be completed in 1 hour with a maximum information density (over the liver) of 250 counts/cm^2. The benefit of the improved statistics achieved by the 3 pulse-height analyzer technique is illustrated in figure 1. If ^{67}Ga is detected in the colon on the first scan, an enema is administered on the same day or on the day following the first scan, and the abdominal region is scanned a second time. If apparent colonic activity is still present, the patient is scanned again on a subsequent day following administration of another enema. Since only the abdomen is re-examined, rescanning time is short, usually less than one-half hour. This method can distinguish fecal activity from tumor involvement (fig. 2).

From autopsy findings, ^{67}Ga is localized initially in the spleen, kidney, adrenal, liver, bone, lymph node, colon, and lung of "normal" human adults (*13*). Scans at 72 hours, however, usually reveal significant concentrations of ^{67}Ga only in the liver, bone, nasopharyngeal area, orbital regions, and, to a lesser extent, the spleen. The typical normal distribution is illustrated in figure 3. Activity in the colon is variable and can often be eliminated by adequate evacuation of feces before scanning. In the absence of disease, ^{67}Ga is only rarely identified in the kidney or lung. Localization of gallium in the lung appears to be more frequent in patients who have undergone lymphography before scanning.

^{67}Ga will also accumulate in any area of inflammation due to infection, mechanical injury, or surgical intervention. We have identified ^{67}Ga activity in the breasts of 3 female patients, 1 of whom was recently pregnant.

Since August 1, 1970, we have performed ^{67}Ga scans on >100 patients with biopsy-proved Hodgkin's disease or other lymphoma. We have restricted our evaluation of data to 24 patients with Hodgkin's disease and 6 patients with malignant lymphoma of the histiocytic type on whom complete staging or restaging, including laparotomy, was carried out, and to 4 additional patients on whom complete staging was not carried out because of preoperative identification of extranodal involvement. All scan interpretations were made by two of us (A.G. and P.B.H.). Diagnosis was made by region, e.g., neck, mediastinum, para-aortic, spleen, etc., and each region was diagnosed as either involved or free of disease. The scan diagnosis was compared with information from available biopsies or, in the case of mediastinal involvement, with chest X-ray, and, occasionally, with mediastinoscopy. These results were previously presented (*4, 5, 14*) and are summarized in tables 1 and 2.

EVALUATION OF DISEASE IN ABDOMEN

The abdomen was the most difficult area to assess by ^{67}Ga scan, the overall accuracy of identifying involved areas being only fair (60%), with a high incidence of false-positive and false-

TABLE 1.—*Accuracy of ^{67}Ga scan in detecting sites of involvement (30 patients, complete staging)*

Site	Positive			Negative		
	Read positive	Read negative	% Error	Read negative	Read positive	% Error
Cervical, supraclavicular and axillary (biopsy proved only)	9	3	25	12	0	0
Mediastinum	13	0	0	15	"2"	12
Total above diaphragm	22	3	12	27	2	7
Para-aortic	4	2	33	19	5	21
Iliac (left and right)	0	4	100	53	3	5
Inguinal (left and right)	1	0	0	57	2	3
Spleen	4	0	0	19	7	27
Total abdomen	9	6	40	148	17	10

TABLE 2.—*Accuracy of ^{67}Ga scan in detecting extranodal sites (excluding liver and spleen) of involvement (34 patients, 30 with complete staging)*

	Positive	Negative
Read positive	5	0
Read negative	0	29
% Error	0	0

negative diagnoses. Certain unique problems exist in evaluating abdominal uptake of ^{67}Ga, and these deserve special attention.

The spleen normally has a greater uptake of ^{67}Ga than surrounding tissues. The spleen, although usually visualized on the 72-hour ^{67}Ga scan, is not prominent in the normal subject. On the posterior scan, ^{67}Ga uptake in the spleen of normal patients is usually less dense than uptake in the liver. When the spleen is enlarged, its nuclide content increases. This is due, at least partially, to the increase in volume of the organ, though increased concentration of the tracer may also play a role. Splenomegaly may occur in patients with Hodgkin's disease with or without splenic infiltration. We have frequently mistaken increased splenic uptake of ^{67}Ga due to benign splenomegaly for ^{67}Ga uptake due to tumor infiltration, and apparently there is no simple method for distinguishing between these 2 causes of increased splenic uptake of ^{67}Ga (*see* fig. 4). In only 1 patient with normal splenic weight and normal histology was increased uptake in the spleen noted.

The para-aortic region is also difficult to evaluate, with a high incidence of false-positive diagnoses. Small amounts of ^{67}Ga in the midline portion of the transverse colon may cause confusion. Furthermore, lordotic curvature of the spine, combined with the "tomographic" effect of the focused collimator, may produce a prominent "lesion" in the anterior view which simulates para-aortic node involvement (*see* fig. 5). We have come to regard all midline abdominal concentrations of nuclide seen on the anterior view as indeterminate unless concentration is marked. If para-aortic uptake of radionuclide is analyzed as asymmetric or midline, and as dense or faint, the asymmetric, dense accumulations are usually due to tumor involvement, and the faint lesions in the midline do not usually indicate disease.

The liver is a prominent area of ^{67}Ga uptake in normal patients. Thus tumor involvement of lymph nodes in the portal region is difficult to identify. Occasionally, however, a lesion in this area may be so prominent that it can easily be detected.

^{67}Ga in the cecum and sigmoid colon may obscure iliac nodes. Equally troublesome is our tendency to interpret activity in these regions as colonic, when it is actually due to node involvement (*see* fig. 6). We believe that thorough evacuation of the bowel before scanning is of critical importance. If activity is identified as colonic, this must be confirmed by repeated scanning following cleansing enemas. If the colon cannot be completely cleared, at least movement of such activity along its course should be seen before it is interpreted as ^{67}Ga trapped in the feces, rather than within a tumor.

EVALUATION OF DISEASE ABOVE DIAPHRAGM

The overall diagnostic accuracy of tumor involvement in the mediastinum, supraclavicular, neck, and axillary regions is good (21/24 sites detected), since there is no interference from accumulation of ^{67}Ga in normal organs. ^{67}Ga uptake, however, is seen in the nasopharyngeal area of normal patients and should not be interpreted as tumor involvement of Waldeyer's ring. Localized uptake of ^{67}Ga also occurs normally in the orbital regions. It has been suggested that this activity is actually concentrated in the lachrymal glands (*15*), but such localization has not been confirmed. Since other centers have not reported uptake of ^{67}Ga on their scans of the orbital region, possibly the uptake of nuclide in this region varies with the method of preparation of the ^{67}Ga-citrate.

EVALUATION OF EXTRANODAL REGIONS

Localization of areas of extralymphatic (excluding liver) infiltration of Hodgkin's disease by ^{67}Ga is accurate (5/5 cases). Frequently such sites have been undetected by other staging tests, and therefore we believe that the *identification of unsuspected Stage IV disease* is the strongest single argument for the use of ^{67}Ga scanning in the staging of patients with Hodgkin's disease or lymphoma (*see* fig. 7).

FUTURE DEVELOPMENTS

The relative success of ^{67}Ga scanning as a supplemental staging procedure for patients with Hodgkin's disease and other malignant lymphoreticular neoplasms encourages further development of the technique. One relatively simple improvement would be to increase the injected dose of radionuclide from the 3 mCi presently in use to the maximum activity that the scanning instrument can efficiently count, probably about 15 mCi. The improved photon yield could be translated directly into an improved image quality, which should enhance our ability to distinguish normal from abnormal structures, especially in the abdomen. The increased radiation dose to the patient would be tolerable, the total-body dose from a 15 mCi injection of ^{67}Ga-citrate being approximately 5 rads. Since the diagnosis of lymphoma has already been established and the patient will probably be receiving a significant radiation dose during therapy, the increased hazard associated with the administration of the additional radionuclide would be small.

Another useful refinement would be a more careful description of tumor localization with regard to lateral position and relative scan density. This is especially important in determining abdominal involvement in the region of the para-aortic nodes. A probability factor could then be assigned to all positive localizations. We currently use such a method informally in our reporting of ^{67}Ga scans, tending to give a high degree of certainty to areas of increased uptake which are not in the midline and show a high concentration of ^{67}Ga and a low degree of probability to apparent lesions which are along the midline and have a relatively low concentration of ^{67}Ga.

Improved scanning equipment may also be helpful. We would prefer a highly efficient detector capable of total-body scanning with tomographic readout in multiple body planes. The tomographic scanner developed by Anger may be suitable for this purpose (*16*). Tomography would be particularly helpful in distinguishing activity in the axial skeleton and transverse colon from para-aortic node localization.

Finally, other agents localizing in tumors should be investigated. ^{111}In-chloride has been suggested for this purpose (*17*). Bismuth-206 is of particular interest, since it demonstrates higher tumor-to-background ratios than any other known agent used for tumor scanning (*18*). Unfortunately, its radiations are difficult to collimate and detect with currently available equipment. Special, large-area GeLi detectors may prove suitable for imaging of this radionuclide (*19*).

CONCLUSIONS

Despite the success we have had with ^{67}Ga-citrate scanning, its nonspecificity and localization characteristics limit its uses. Consequently, the "ideal" agent for scanning lymphoma patients has yet to be developed.

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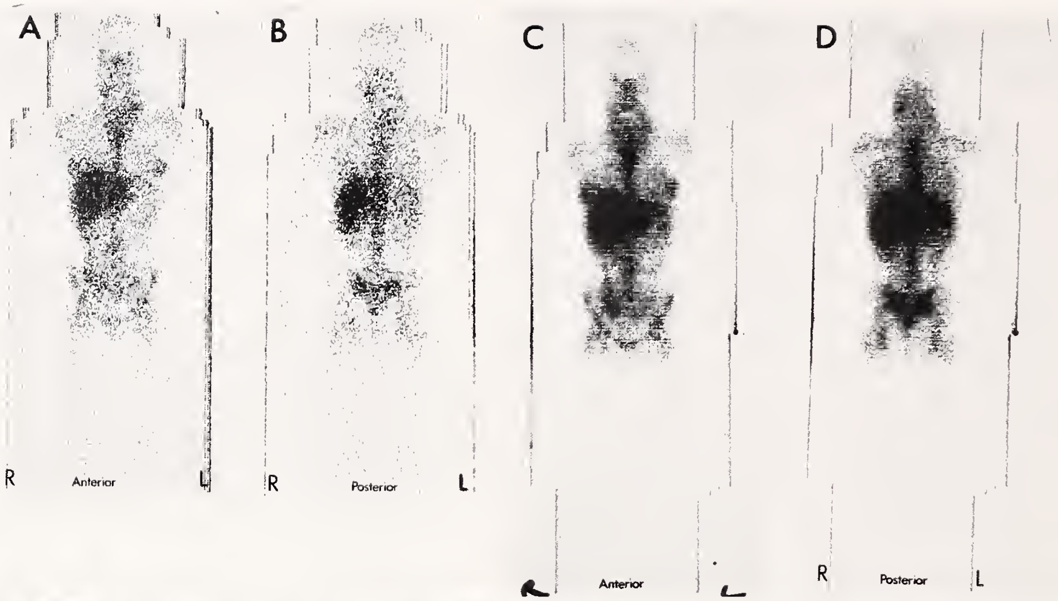

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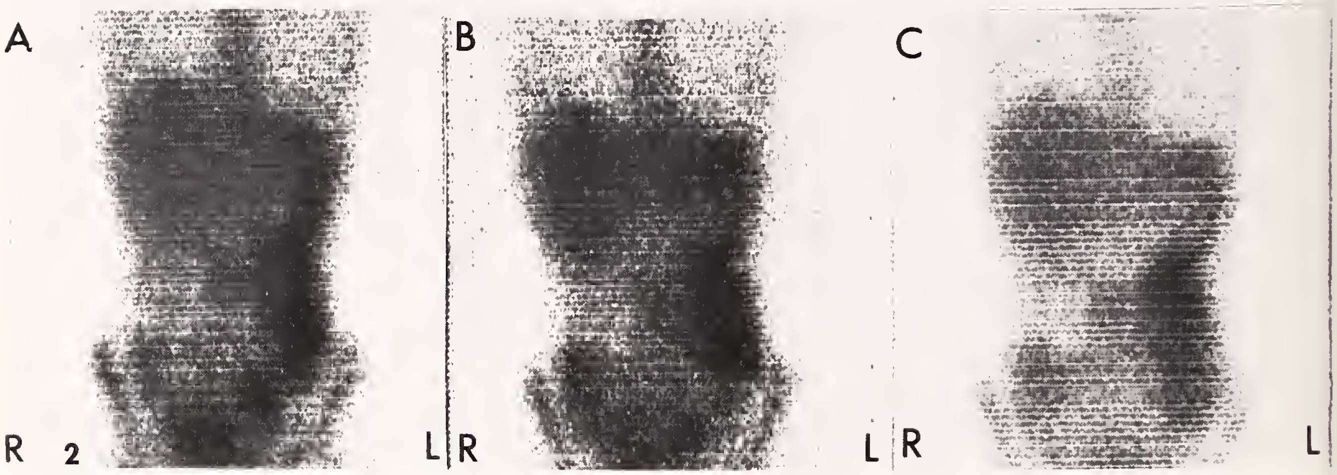


2

FIGURE 1.—*A* and *B:* Low information density scan (50 counts/cm^2) produced by scanning with the 296 Kev peak of ^{67}Ga. Localization of tumor involvement is difficult. *C* and *D:* High information density scan (230 counts/ca^2) produced by simultaneously scanning the 94 kev, 183 keV, and 296 kev peaks of ^{67}Ga (3 independent pulse height analyzers). Tumor involvement in mediastinum, neck, and right iliac region can now be more readily identified. Scan time is the same in both sets of scans. *Note also* breast activity and possible para-aortic node involvement.

FIGURE 2.—*A, B, C:* Apparent localization of ^{67}Ga in feces (*A*). However, following two attempts at evacuation with enemas, activity remains (*B* and *C*). This strongly suggests tumor involvement rather than fecal localization.

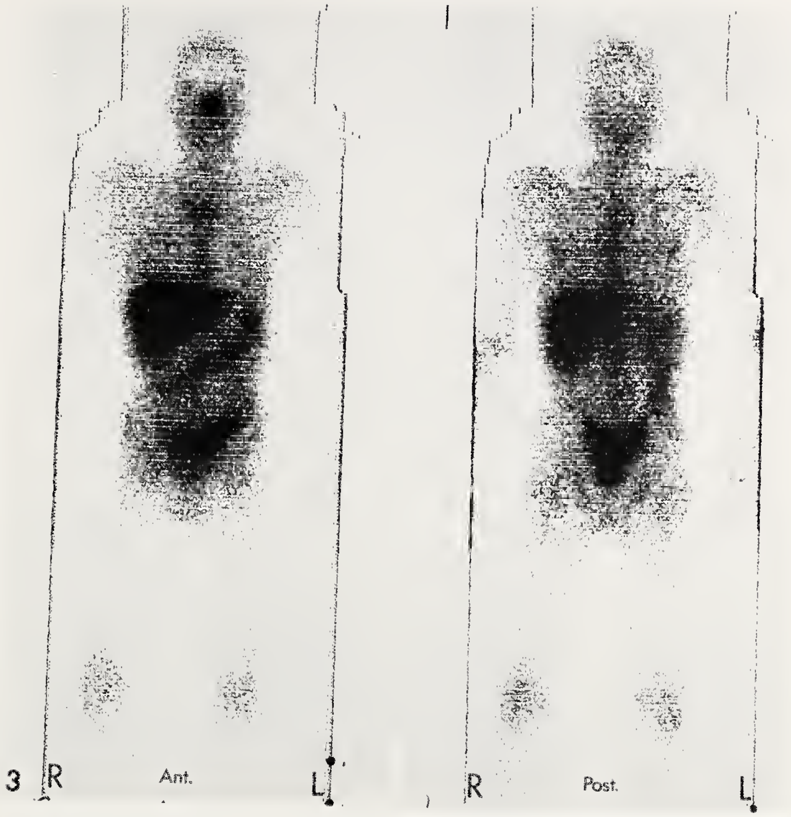
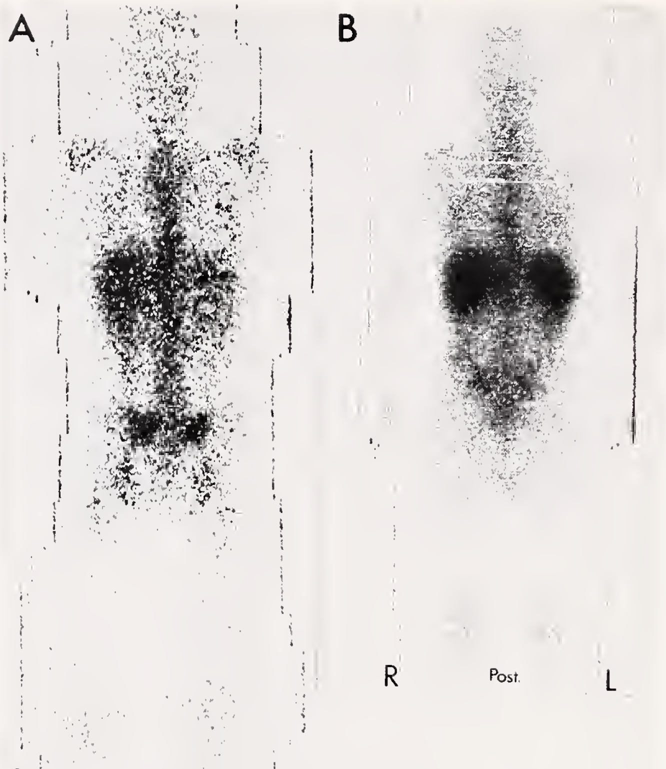


Figure 3.—Normal anterior and posterior ^{67}Ga scan 72 hours after intravenous injection of 3 mCi of ^{67}Ga-citrate. *Note* activity in nasopharynx, bone, liver, and colon.



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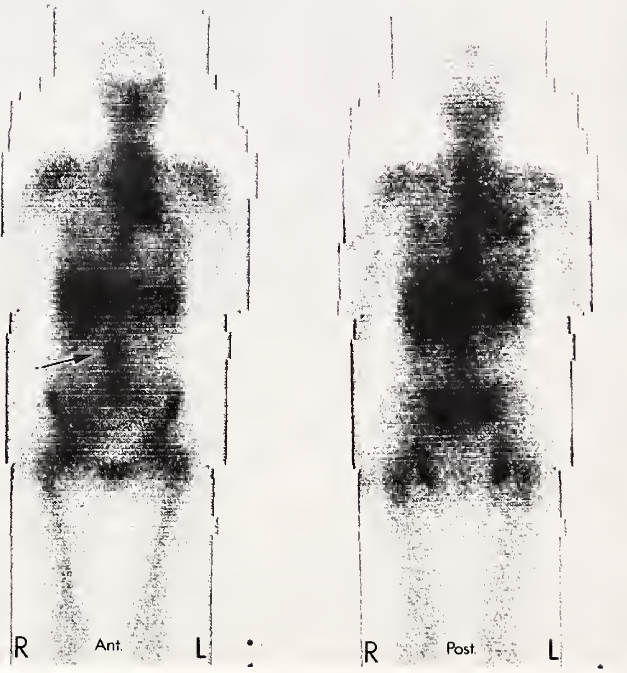

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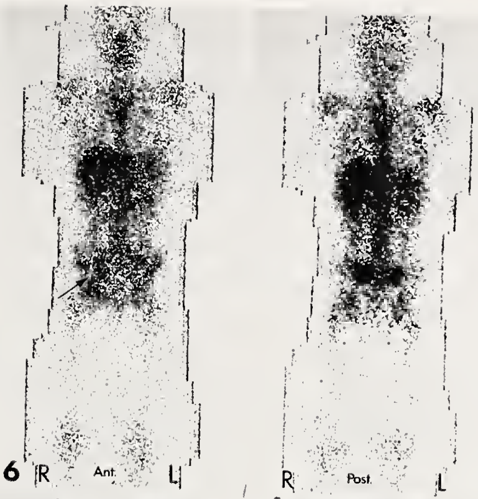
FIGURE 4.—*A:* Increased splenic uptake due to lymphomatous involvement of the spleen. On the posterior view shown here, the spleen is almost as "hot" as the liver. In normal patients, posterior view of liver is denser than posterior view of spleen. *B:* Increased splenic uptake due to "benign" splenomegaly. No lymphomatous involvement of the spleen was demonstrated by pathologic examination.

FIGURE 5.—Prominent lordotic curvature simulating para-aortic node involvement (*arrow*) in the anterior view. Cervical, mediastinal, splenic right inguinal involvement correctly identified (recent case, not included in table 1).

6



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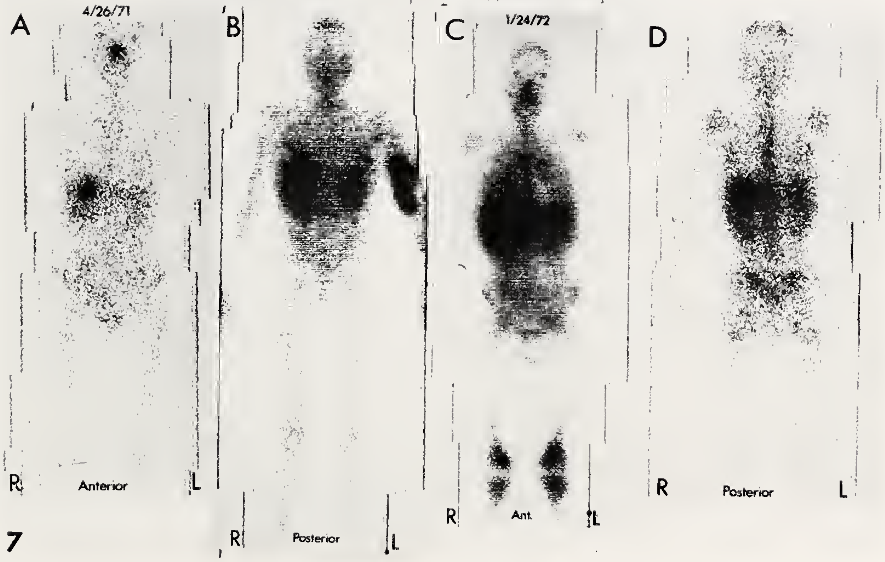

FIGURE 6.—Iliac node involvement. Activity in the right lower quadrant of the abdomen (*arrow*) was misinterpreted as representing ^{67}Ga accumulation in feces in cecum.

FIGURE 7.—*A:* Chest wall involvement. Restaging evaluation was otherwise normal. Biopsy of this region revealed tumor infiltration. *B:* Tumor infiltration of left arm. Before the scan, arm swelling was thought to be lymphedema due to lymphatic obstruction in axilla. Biopsy of arm lesion revealed tumor; axilla and spleen also positive. *C:* Severely ill patient with presumed Stage IV Hodgkin's disease. Unsuspected lesion in left knee proved most convenient site to biopsy to establish extranodal involvement. Splenic, chest, mediastinal, and right inguinal lesions are also seen. *D:* Splenic bed involvement. Restaging workup demonstrated uptake of ^{67}Ga in splenic bed. However, splenectomy had been previously performed. Second lapartomy revealed large tumor mass in splenic bed.

Invited Discussion: Value of Radiogallium Scanning in Hodgkin's Disease [1]

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THE DIFFICULTY of detecting hepatic and splenic involvement by methods other than direct histologic examination of these tissues has led to the introduction and widespread adoption of exploratory laparotomy and splenectomy as a staging procedure in Hodgkin's disease. Despite pathologic staging, the appearance of extranodal disease in some patients after radical irradiation indicates that undetected occult foci of disease were present initially, and an isotope which concentrates in Hodgkin's tissue would be an important addition to the assessment and management of the disease. With these considerations in mind, we are assessing, in collaboration with Dr. V. R. McCready in the Nuclear Medicine Department, the possible value of ^{67}gallium (^{67}Ga) in Hodgkin's disease (*1*).

We have scanned 46 patients and correlated the scan with the clinical findings as a preliminary measure so that some reliance can be placed subsequently on the ability of the technique to detect occult disease. Our major effort has been directed toward supradiaphragmatic disease, since we have found that the interpretation of infradiaphragmatic scans is complicated by colonic excretion, as described by Hoffer et al. (*2*). Of 46 patients, 31 (67%) showed either complete concordance of the clinical assessment and scan or uptake in most of the involved areas (table 1). In the latter group, where uptake could not be demonstrated in all clinically involved areas, a positive scan was related to the mass of Hodgkin's tissue present. In bilateral neck disease, the scan tended to be positive on the side bearing bulky nodes, whereas no uptake could be demonstrated in the contralateral smaller node mass. Agreement between the scan and clinical findings was related to histologic grade. In patients with nodular sclerosis (NS), 19/24 patients (79%) showed either good or partial agreement, compared with 8/17 (47%) in mixed-cellularity (MC) disease.

TABLE 1.—^{67}*Ga scanning in Hodgkin's disease, The Royal Marsden Hospital*

	Histologic grade*	Clinical/scan correlation		
		Good	Partial	Poor or none
Untreated patients	LP	2	0	1
	NS	4	6†	4‡
	MC	3	3	2
	LD	1	1	0
Recurrent cases	NS	8	1	1
	MC	0	2	7
		18	13	15

* LP=lymphocyte predominant; and LD=lymphocyte depletion.

† 3 patients had positive mediastinal scan with negative radiograph.

‡ In 3 patients, mantle irradiation had been started before scanning.

In our limited experience, ^{67}Ga scanning has effectively demonstrated pulmonary infiltration, but this is usually obvious on the chest radiograph. In one patient, however, who showed uptake in the right lower lobe and in whom the chest radiograph was initially normal, radiologic evidence of pulmonary infiltration subsequently appeared at this site. Three patients with NS and bilateral neck nodes with negative chest radiographs showed ^{67}Ga uptake in the mediastinum. The scan was negative after mantle irradiation and it may be inferred, but not proved, that the mediastinal lymph nodes were involved, though they were not apparent radiologically.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

In 3 patients with NS and mediastinal disease, supradiaphragmatic scanning, which proved to be negative, was not performed until mantle irradiation had been started. Since mediastinal uptake has been seen almost invariably in this group, the negative results suggest that ^{67}Ga uptake may be abolished by radiation doses of $<$1000 R.

Uptake in the humeral epiphyses in children and young adults may be confused with axillary node disease, and midline uptake, which is probably esophageal, with mediastinal disease. We have seen pulmonary uptake in a patient with a lower lobe pneumonia.

So far, the non-Hodgkin's lymphomas have not been studied systematically, but we have observed uptake in reactive hyperplasia, lymphocytic lymphoma, and histocytic lymphoma.

One immediate diagnostic problem where ^{67}Ga appears to be of value is the distinction between postirradiation pulmonary fibrosis and mediastinal recurrence. A sequence of repeat investigations showing a positive scan before treatment, a negative scan after completion of irradiation, and a persistently negative scan when radiologic lung changes supervene provides useful supportive evidence of fibrosis rather than recurrence.

In summary, ^{67}Ga scanning in Hodgkin's disease appears to be a promising investigative method which needs a fuller evaluation, especially with sequential scans, before its value can be adequately assessed. So far, in our experience, there has been a reasonably good correlation between extent of disease and isotope uptake, though small but histologically positive cervical or axillary nodes may not be detected. We are currently exploring possible means for improving the resolution of the method, sufficient to permit the detection of occult extranodal disease.

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SUMMARY OF INFORMAL DISCUSSION ON RADIOGRAPHIC AND ISOTOPIC DIAGNOSIS

D. W. Smithers asked whether the data concerning "false-negative" and "false-positive" lymphangiograms are not subject to doubt because of surgical sampling error in obtaining the lymphangiographically suspicious lymph nodes. **R. Castellino** replied that this is a problem, but that he had routinely circled the suspicious nodes preoperatively and then compared these films with the location of the metal clips placed at the biopsy site (s), as revealed on postoperative films. Cases in which there was an obvious discrepancy had not been scored as false positives.

J. E. Ultmann commented on the additional value which the metal clips may have in some instances when their change in position or separation reveals enlarging para-aortic nodes. **P. B. Hoffer** stated that gallium scans may also be useful in such cases. **H. S. Kaplan** asked whether differential clearance rates in Hodgkin's disease-involved tissues and in normal liver and colon might be exploited to overcome the difficulties in abdominal scanning. He also asked whether the endolymphatic route had been used with ^{67}Ga. **Hoffer** replied that the group at the Oak Ridge National Laboratory had recently published data on differential clearance rates, but clinical attempts to utilize this approach had not been carried very far. **Castellino** asked whether gallium scans could offer any help with a difficult and frequent problem, the differential diagnosis of infection versus Hodgkin's disease in pulmonary infiltrates. **Hoffer** said that he did not think gallium would be differentially taken up in such situations and would therefore offer little help.

G. R. Ray commented that the results of gallium scanning at Stanford University are still very preliminary. The problem with gallium scanning is that it is not tumor specific in terms of detecting Hodgkin's disease. Moreover, the procedure has certain hazards, such as bone marrow radiation exposure. Yet, this scanning agent is being used routinely now in many non-university centers in the United States before it has really been established as a reliable method. One sees patients who, even before their biopsy diagnosis is confirmed, have already had a ^{67}Ga scan and a diagnosis made from the gallium scan irrespective of the biopsy. It is only after the biopsy is read that the gallium scan is reviewed and reinterpreted.

The total experience at Stanford University relates to scans performed with ^{67}Ga in 19 patients with Hodgkin's disease and in 45 other patients with other types of tumors. There were 38 sites that were clinically evident in the 19 patients with Hodgkin's disease. Positive uptakes in definitely involved sites, or "true positives," were noted in 26 of 38 sites (68%) in 11 of 19 patients (60%). False positives are those in which the scan showed increased activity in areas without clinically evident disease. This occurred in 2 patients at 2 separate sites, a false positive rate of 11%. Of greater concern is the incidence of false negatives; these false negatives occurred in 12 of 38 sites (32%) in 8 of 19 patients, or 40%—the same figure that **Doctor Hoffer** mentioned. Occult disease was detected in 3 of 19 patients (16%). In 2 of these patients, this was infraclavicular node disease which was not felt on physical examination. One other patient had an extranodal site of uptake in bone which was not detected on routine X-ray examinations. Subsequently the patient had a radiostrontium scan which showed increased activity over that area, and laminograms showed changes consistent with Hodgkin's disease, but this lesion was not biopsy-proved.

In collaboration with **Doctors Horn** and **Kriss, Doctor Ray** has also attempted to analyze the postlaparotomy biopsy material with respect to the concentration of gallium, expressed as percentage of injected dose per gram of tissue. To date, this has been done in only a few patients. The spleen and lymph nodes were taken directly from the operating room and counted in a well counter with standard geometry and with a reference aliquot of the injected dose. Thus the amount in the tissue can be expressed as a percentage of the injected dose. In one patient with nodular-sclerosing Hodgkin's disease, there was a marked difference between a noninvolved node and a histologically positive node. A similar observation was made in another patient who had mixed-cellularity Hodgkin's disease; this patient had focal involvement in one para-aortic node, and there was significantly less activity in this node than in a second node which was diffusely involved. In a patient with nodular sclerosis involving the spleen, **Doctors Horn, Kriss,** and **Ray** compared normal areas with grossly involved areas containing nodules. There was a distinct difference (roughly twofold) between the grossly uninvolved and the grossly involved areas of the same spleen. However, histologic examination of the pathologic specimen revealed that both specimens had tumor in them, and, therefore, there really should have been no difference.

Staging Laparotomy and Splenectomy in Hodgkin's Disease: Analysis of Indications and Patterns of Involvement in 285 Consecutive, Unselected Patients [1,2]

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SUMMARY—The frequency of subdiaphragmatic involvement in a series of 285 consecutive, unselected, previously untreated patients with Hodgkin's disease, of whom 272 underwent staging laparotomy with splenectomy, is analyzed with respect to the influence of age, sex, histopathologic type, preoperative anatomic sites of involvement, constitutional symptoms, lymphangiographic findings, and other factors. From these data, low- and high-yield groups may be identified, providing a basis for the selection of patients with Hodgkin's disease in whom staging laparotomy is indicated. Diagnostic information made available by laparotomy with splenectomy and biopsy resulted in a change of stage in 18% and influenced treatment decisions in 35% of all cases.—Natl Cancer Inst Monogr 36: 291–301, 1973.

LAPAROTOMY WITH splenectomy and biopsy of the liver and abdominal lymph nodes for the evaluation of selected patients with Hodgkin's disease has been used intermittently at Stanford University Medical Center since 1960. By 1968, a series of 65 selected cases had been collected in which a surprisingly high incidence of clinically unsuspected spleen involvement, an appreciable frequency of microscopic involvement of the para-aortic nodes, and a distinctly nonrandom association between spleen and liver involvement were observed (*1*). The technical aspects of these surgical diagnostic procedures (hereinafter collectively termed a "staging laparotomy" for brevity and convenience) were described by our surgical colleagues (*2*), who reported a nil mortality and an acceptably low postoperative morbidity in this initial series of patients. Several other centers were stimulated to use staging laparotomies in patients with Hodgkin's disease and have reported similar results (*3–6*).

It was clear, however, that valid estimates of the frequency of involvement of the spleen, liver, and lymph nodes, and of their association with other parameters such as histopathologic type, various supradiaphragmatic sites of involvement, and constitutional symptoms could only be obtained from a large-scale study in which consecutive, *unselected* patients with biopsy-proved, untreated Hodgkin's disease were routinely evaluated with the aid of staging laparotomy, as well as the other diagnostic procedures we have used in the past (*7*). Such a study was undertaken at Stanford in the summer of 1968 and has been continued uninterruptedly to the present date. Interim reports of the findings in the first 50 and 100 consecutive, unselected cases have been published (*7, 8*), as have correlations of the histopathologic and clinical features in combined series of selected and unselected patients (*9, 10*). However, larger numbers of cases were required to

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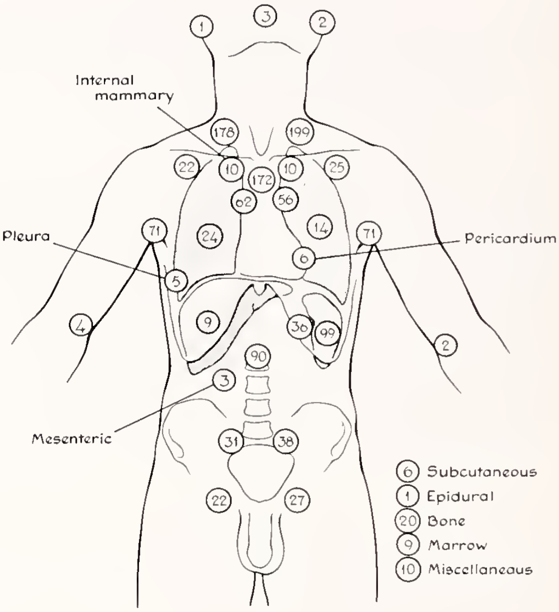
provide reasonable estimates of the probability of subdiaphragmatic involvement in patients with different patterns of clinical presentation.

This paper presents the first such analysis, based on a series of 285 consecutive unselected, previously untreated, biopsy-proved cases of Hodgkin's disease admitted to Stanford University Medical Center between mid-1968 and December 31, 1971. Thirteen patients did not have staging laparotomy—twelve because of preoperatively documented Stage IV disease involving the bone marrow, multiple bones, or both lungs, and one due to advanced age and infirmity. Laparotomy was performed on 172 males and 100 females. The ages of these patients ranged from 21 months to 65 years, with a peak age in the 21- to 30-year-old bracket. The histopathologic findings in the spleen, wedge and needle liver biopsies, lymph node biopsies, and open bone-marrow biopsies routinely obtained in the 272 laparotomized cases have been related to age, sex, histopathologic type, preoperative anatomic sites of involvement, and other factors in an attempt to provide data on the basis of which the indications for selective use of staging laparotomy might be formulated.

RESULTS

The overall frequency of involvement of various anatomic sites in all 285 patients is illustrated in text-figure 1. The observed distributions were similar to those recorded in an earlier study (*11*) of 340 patients in whom staging laparotomy had not been routinely used, except, as might have been anticipated, for the significantly greater frequency of spleen involvement in the new study. Whereas there were 44 instances (13%) of clinically apparent spleen involvement among the earlier 340 patients, there were 99 histologically verified positive spleens among the new series of 272 routinely laparotomized cases (36%). As noted previously (*8–10*), the probability of splenic involvement is a function of the Rye histopathologic type, increasing from 12% in lymphocytic predominance (LP) and 34% in nodular sclerosis (NS) to over 50% in the mixed-cellularity (MC) and lymphocytic depletion (LD) types (table 1). Similarly, para-aortic node involvement was documented in 19% of cases of LP predominance, 34% of cases of NS, and 44% of cases of MC type; there were only 2 cases of the LD type (a third case thus diagnosed initially was later reclassified as an anaplastic neoplasm), in both of which spleen and para-aortic node involvement was present.



TEXT-FIGURE 1.—Hodgkin's disease showing the anatomic distribution of sites of involvement in 285 consecutive, unselected, previously untreated cases (285 cases, 272 laparotomies).

These relationships account in large part for the observed differences in Ann Arbor pathologic stage (*12*) distributions among the Rye histopathologic types (table 2). It may be noted that 21/26 cases (81%) of LP and 119/206 cases (58%) of NS were assigned after laparotomy to pathologic Stages I or II, whereas 27/49 (55%) of MC cases and both of the LD cases were of pathologic Stage III or IV extent. It is also of interest that all but one of the 38 cases with localized extralymphatic (II$_E$ or III$_E$) involvement were of the NS type; in contrast, extralymphatic involvement in cases of MC type tended to occur in the liver and/or bone marrow and was thus classified in pathologic Stage IV.

TABLE 1.—*Histopathologic type versus frequency of para-aortic and splenic involvement in 272 patients with staging laparotomy*

Histo-pathologic type	Cases*	Para-aortic nodes positive		Spleen positive	
		Number	Percent	Number	Percent
LP	26 (9.6)	5	19	3	12
NS	194 (71.3)	66	34	67	34
MC	48 (17.6)	21	44	27	56
LD	3 (1.1)	2	67	2	67
Unclassified	1 (0.4)	0	—	0	—
	272 (100)	94	34	99	36

* Number of cases of each type submitted to laparotomy and (% of total series).

TABLE 2.—*Pathologic stage distribution versus histopathologic type*

Histo-pathologic type (Rye)	I-A	Pathologic stage (Ann Arbor)*												
		I-B	II-A	II-B	$\mathrm{II_E}$ (A+B)	III-A	III-B	$\mathrm{III_E}$ (A+B)	$\mathrm{III_S}$-A	$\mathrm{III_S}$-B	$\mathrm{III_{ES}}$ (A+B)	IV-A	IV-B	Total
LP	9	0	11	1	0	3	0	0	1	0	0	1	0	26
NS	16	2	47	29	25	6	1	4	24	19	8	3	22	206
MC	3	0	6	9	1	1	0	0	17	5	0	1	6	49
LD	1†	0	0	0	0	0	0	0	0	2	0	0	0	3
Unclassified		I	0	0	0	0	0	0	0	0	0	0	0	1
Total	0	3	64	39	26	10	I	4	42	26	8	5	28	285*

* The pathologic stage was based on laparotomy findings in 272 patients.

† This case was later reclassified, after biopsy of massive retroperitoneal metastasis, as an anaplastic malignancy, most probably diffuse histiocytic lymphoma.

The tissue specimens provided by laparotomy yielded histologic evidence which altered the final pathologic stage (PS), relative to the prelaparotomy clinical stage (CS), in 50 of 272 patients (18.2%), in all but 2 of which the final stage was increased (table 3). Eight of thirty-six cases (22%) originally thought to be of CS I-A extent were reclassified, with the aid of laparotomy findings, as PS III-A; reclassification to PS III (A or B, respectively) also occurred in 21 of 99 (21%) CS II-A or $\mathrm{II_E}$-A cases and in 11 of 57 (19%) CS II-B or $\mathrm{II_E}$-B cases. Among the 33 CS III-A or $\mathrm{III_E}$-A cases, there was a shift up to PS IV-A or down to PS II-A in one case each, whereas shifts up to PS IV-B (7 cases) or down to PS II-B (1 case) were more frequent among the 36 CS III cases with constitutional symptoms (III-B or $\mathrm{III_E}$-B). In addition to those cases in which a change of stage resulted, laparotomy documented the presence of spleen involvement in 22 of 33 (67%) CS III-A and in 25 of 36 (69%) CS III-B cases. Inasmuch as the presence or absence of spleen involvement has been one of the criteria determining our treatment policy (*13, 14*), it may be concluded that laparotomy yielded information of vital importance for the subsequent management of patients in 97 of 272 cases (35%).

The presence or absence of lymphangiographic evidence of para-aortic node involvement was strongly correlated with the frequency of positive para-aortic node biopsies (table 4). The overall frequency of "false-negative" interpretations

TABLE 3.—*Influence of laparotomy findings on final stage*

Initial clinical stage (Ann Arbor)	Postlaparotomy pathologic stage			Total No. of cases	Change of stage	
	Unchanged	Increased	Decreased		Number	Percent
I-A	28	8	—	36	8	22
I-B	3	0	—	3	0	—
II-A, II$_E$-A	78	21	0	99	21	21
II-B, II$_E$-B	46	11	0	57	11	19
III-A, III$_E$-A*	31	1	1	33	2	6
III-B, III$_E$-B*	28	7	1	36	8	22
IV-A	2	—	0	2	0	—
IV-B	6	—	0	6	0	—
Total	222	48	2	272†	50	18.2

* Laparotomy revealed spleen involvement in 22/33 CS III-A and in 25/36 CS III-B cases. When these cases, reclassified within Stage III as PS III$_S$-A and PS III$_S$-B, respectively, are combined with those in which a change of stage was documented, laparotomy is seen to have yielded significant additional information in 97/272 cases (35%).

† Laparotomies were not performed on 13 other patients (12 patients in Stage IV and 1 patient of advanced age).

was only 5 of 142 (3.5%). Of these, 2 of 19 (11%) were of the MC type, which suggests that a negative lymphangiogram is least reliable in this type of Hodgkin's disease; in contrast, there were only 3 false-negative cases among 103 of the NS type, and none among 18 of the LP type. The frequency of unsuspected spleen involvement in patients with a negative lymphangiogram followed a similar pattern; there were 7 positive spleens in the 19 (37%) cases with MC histology, 13 positive spleens in the 103 (13%) cases with NS histology, and 0 positive spleens in the 18 cases with LP histology (or among the 3 additional cases of this histologic type in which the lymphangiogram was equivocal). Thus, apparently, patients with the LP type of Hodgkin's disease in whom the lymphangiogram is negative or equivocal may constitute one clinical subcategory in which staging laparotomy could safely be omitted. In contrast, the yield of positive spleens in those with the other histologies and a negative or equivocal lymphangiogram is too great, in our opinion, to warrant the omission of laparotomy; there were 22 positive spleens among 137 cases of NS (16%) and 12 positive spleens among 30 cases of MC (40%).

As might have been expected, there were much more positive para-aortic node biopsies and spleen specimens among patients with a positive lymphangiogram, regardless of histology. The relatively high "false-positive" error (24%) of lymphangiographic interpretation was due in part to the problems of surgical sampling of the suspicious lymph nodes and in part to the occurrence of other abnormalities (reactive hyperplasia, epithelioid or other granulomas) which may simulate the radiographic changes induced in the para-aortic nodes by lymphomatous invasion (*1, 15*). Almost all patients with a positive lymphangiogram and positive para-aortic node biopsy had positive spleens, except 3 patients with LP histology whose node biopsies were positive but whose spleens were negative. Conversely, several patients with NS or MC histology and positive lymphangiograms had positive spleens despite negative para-aortic node biopsies.

Analyses of the influence of age and sex on the yield of positive staging laparotomy findings are presented, in relation to preoperative lymphangiographic interpretation, in tables 5 and 6. Except for a slightly lower yield in the 21- to 30-year age group, which may reflect the greater prevalence of NS at these ages, the data

TABLE 4.—*Laparotomy findings in relation to histopathologic type and lymphangiographic interpretation*

LAG*	Histopathologic type	Para-aortic node biopsy						Spleen				
		Positive	Negative	ND*	Total	Percent Positive	Percent error	Positive	Negative	ND	Total	Percent positive
+	LP	5	0	—	5	100	0	2	3	—	5	40
	NS	40	16	—	56	71	29	42	13	1	56	76
	MC	14	3	1	18	82	18	17	1	—	18	94
	LD	2	0	—	2	100	0	2	0	—	2	100
	Subtotal	61	19	1	81	76	24	63	17	1	81	79
±?	LP	0	3	—	3	0	0	0	3	—	3	0
	NS	4	30	—	34	12	12	9	25	—	34	26
	MC	1	10	—	11	9	9	5	6	—	11	45
	LD	—	—	—	0	—	—	—	—	—	0	—
	Subtotal	5	43	0	48	10	10	14	34	0	48	29
—	LP	0	18	—	18	0	0	0	18	—	18	0
	NS	3	100	—	103	3	3	13	90	—	103	13
	MC	2	17	—	19	11	11	7	12	—	19	37
	LD	0	1	—	1	0	0	0	1	—	1	0
	Unclassified	0	1	—	1	0	0	0	1	—	1	0
	Subtotal	5	137	0	142	3.5	3.5	20	122	0	142	14
	Total	71	199	1	271†	26	10.7	97	173	1	271	36

* LAG = lymphangiogram interpretation; + = positive for para-aortic node abnormality; ±? = equivocal; — = negative. ND = tissue sample not obtained.

† One patient with a positive laparotomy (spleen and nodes) did not have lymphangiography due to a history of allergic reactions to organic iodide compounds.

were surprisingly uniform from the second through the sixth decade (table 5). The numbers of cases in the 0- to 10-year and 61+-year age groups were too small to give great weight to the low incidence of para-aortic node involvement observed in the former, or of spleen involvement in the latter. Interestingly, however, 6 of 11 cases (55%) in the 0- to 10-year age group had positive spleens, 4 of which were clinically unsuspected. Thus, the pediatric age group is by no means free of the hazard of silent spleen involvement. When the data of the first 3 decades were combined and compared with those of the older groups, highly significant differences in the frequency of positive lymphangiograms and of positive para-aortic node biopsies became apparent. Whereas lymphangiograms were positive in only 44 (24.7%) and para-aortic node biopsies in 33 (18.5%) of 178 young-adult, adolescent, and pediatric cases, the corresponding data for the 92 older cases were 36 (39.1%) and 30 (32.6%), respectively ($P<0.01$). Thus, the unfavorable prognosis of older patients with Hodgkin's disease may be due, at least in part, to their greater propensity for retroperitoneal node involvement, usually associated with disease of Stage III or IV extent.

Para-aortic node biopsy was positive in 53 (31%) of 170 males and in only 20 (20%) of 100 females, a significant difference at the 5% level (table 6). Spleen involvement was also somewhat more frequent among males (67/171, or 39%) than among females (29/99, or 29%), but this difference was not significant. Overall, the age and sex data provide no selective indications relevant to staging laparotomy; clearly it would not be safe to omit this diagnostic procedure in either sex, nor in any age group during the first 6 decades, since even patients $\geqq 51$ years of age with negative lymphangiograms had a distinctly non-negligible yield of positive spleens (3/12, or 25%).

Analysis of our laparotomy data in relation to preoperative sites of lymphadenopathy reveals that there were no positive spleens or para-aortic node biopsies among 11 patients with CS I or II disease apparently confined to the thorax (mediastinal nodes and/or thymus, alone or accompanied by hilar node, pulmonary parenchymal, or pericardial involvement) or among 5 patients with CS I disease apparently confined to 1 axilla (table 7). Positive laparotomy yields were thus concentrated among those patients whose lymphadenopathy presented either in the groin or the cervical-supraclavicular region. Involvement of the left cervical-supraclavicular region, alone or associated with mediastinal or other supradiaphragmatic sites, was accompanied by para-aortic or splenic hilar node disease more often than right cervical-supraclavicular involvement, but the differences were somewhat less striking than those noted in our earlier reports (*1, 7, 8*). Moreover, no such difference in laparotomy yield relative to left versus right cervical-supraclavicular adenopathy has been noted in the data of certain other institutions (*5, 6, 16*). When the presence and location of cervical-supraclavicular adenopathy were further analyzed with respect to both histologic type and lymphangiogram interpretation (table 8), all 4 positive para-aortic node biopsies associated with right-sided cervical-supraclavicular involvement were noted to have had NS histology. Moreover, there were no positive spleens among the 5 patients with LP histology clinically confined to the right side of the neck. However, as was indicated above, the relevant indication with this histologic type would appear to be the lymphangiographic interpretation, since there were no positive laparotomy findings, regardless of left, right, or bilateral cervical-supraclavicular adenopathy, when the lymphangiogram was negative or equivocal. Although none of the 8 cases of MC disease limited to the right side of the neck (alone or with ipsilateral axillary involvement) had positive para-aortic node biopsy, 3 of 8 had a clinically unsuspected positive spleen.

Summing up the influence of other sites of involvement, it is suggested that staging laparotomy can safely be omitted in patients whose Hodgkin's disease is confined to the mediastinal nodes, with or without hilar or pulmonary involvement, provided that the absence of low cervical-supraclavicular adenopathy is ascertained with particular care, with scalene node biopsy in doubtful cases. Although the present data suggest that patients with CS I unilateral axillary disease may also have a nil probability of positive laparotomy findings, such cases are

TABLE 5.—*Influence of age on laparotomy findings*

		Para-aortic node biopsy	Spleen
Age (yr)	LAG*	Number positive/ total No.	Number positive/ total No.
0–10	+	1/ 1†	2/ 2
	±?	0/ 2	1/ 2
	—	0/ 7	3/ 7
	Subtotal (%)	1/10 (10%)	6/11 (55%)
11–20	+	12/14	12/13‡
	±?	2/15	4/15
	—	1/33	8/33
	Subtotal (%)	15/62 (24%)	24/61 (39%)
21–30	+	20/29	23/29
	±?	1/20	2/20
	—	2/57	4/57
	Subtotal (%)	23/106§ (22%)	29/106§ (27%)
31–40	+	15/19	14/19
	±?	1/10	3/10
	—	0/18	1/18
	Subtotal (%)	16/47 (35%)	18/47 (39%)
41–50	+	5/ 6	4/ 6
	±?	—	—
	—	1/15	4/15
	Subtotal (%)	6/21 (29%)	8/21 (38%)
51–60	+	7/ 7	6/ 7
	±?	1/ 1	1/ 1
	—	0/10	2/10
	Subtotal (%)	8/18 (44%)	9/18 (50%)
61+	+	3/ 4	0/ 4
	±?	—	—
	—	1/ 2	1/ 2
	Subtotal (%)	4/ 6 (67%)	1/ 6 (16%)

* LAG = lymphangiogram interpretation: + = positive for para-aortic node abnormality; ±? = equivocal; — = negative.

† Para-aortic node biopsy not obtained in 1 case.

‡ Splenectomy not performed on 1 patient.

§ One patient in this age group, with a positive laparotomy (nodes and spleen), had no prior lymphangiography because of a strongly allergic history.

TABLE 6.—*Influence of sex on laparotomy findings*

Sex	LAG*	Para-aortic node biopsy	Spleen
		Number positive/total No.	Number positive/total No.
Male	+	46/54 (2)†	43/55
	±?	3/28 (1)	9/28
	−	4/88 (4)	15/88
	Total	53/170 (31%)	67/171 (39%)
Female	+	18/28 (4)	21/27‡
	±?	1/18	3/18
	−	1/54(1)	5/54
	Total	20/100 (20%)	29/99 (29%)

*LAG = lymphangiogram interpretation: + = positive for para-aortic node abnormality; ±? = equivocal; − = negative. One female patient with a positive laparotomy (nodes and spleen) had no prior lymphangiography because of a strongly allergic history.

†Para-aortic node biopsy was not obtained in 1 case. Numbers in parentheses indicate cases with positive splenic hilar nodes and negative para-aortic node biopsy.

‡Splenectomy was not performed on 1 patient.

TABLE 7.—*Association of subdiaphragmatic involvement at laparotomy with various preoperative patterns of lymphadenopathy**

Preoperative sites of involvement	Total No. of cases	Laparotomy findings			
		Para-aortic/splenic hilar nodes positive		Spleen positive	
		Number	Percent	Number	Percent
Mediastinal nodes only	11	0	—	0	—
Unilateral axillary nodes only	5	0	—	0	—
Cervical-supraclavicular nodes only:					
Left	26	10	38	9	35
Right	13	1	8	3	23
Bilateral	13	6	46	5	38
Cervical-supraclavicular and mediastinal nodes only:					
Left	24	6	25	7	29
Right	15	2	13	2	13
Bilateral	42	16	38	17	40
Cervical-supraclavicular nodes, alone or with other supra-diaphragmatic sites:					
Left	68	22	32	24	35
Right	44	8	18	9	20
Bilateral	126	57	45	59	47
Inguinal, femoral, and/or iliac nodes only	16	8	50	5	31

*285 consecutive previously untreated cases, of which 272 were submitted to staging laparotomy.

TABLE 8.—*Laparotomy findings in relation to cervical-supraclavicular node involvement, by histologic type and lymphangiogram result*

Histologic type	Neck nodes	Para-aortic nodes* (Number positive/total No.)				Spleen* (Number positive/total No.)			
		LAG +	LAG ±?	LAG −	Total	LAG +	LAG ±?	LAG −	Total
LP	Right	—	—	0/ 5	0/ 5	—	—	0/ 5	0/ 5
	Left	4/ 4	0/ 1	0/ 6	4/11	2/ 4	0/ 1	0/ 6	2/11
	Bilateral	—	0/ 1	0/ 5	0/ 6	—	0/ 1	0/ 5	0/ 6
	None	1/ 1	0/ 1	0/ 2	1/ 4	0/ 1	0/ 1	0/ 2	0/ 4
	Subtotal	5/ 5	0/ 3	0/18	5/26	2/ 5	0/ 3	0/18	2/26
	Percent	100	0	0	19	40	0	0	8
MC	Right	—	0/ 4	0/ 4	0/ 8	—	1/ 4	2/ 4	3/ 8
	Left	8/10(1)	0/ 6	0/ 5	8/21(1)	10/10	2/ 6	1/ 5	13/21
	Bilateral	5/ 6	1/ 2	2/ 7	8/15	5/ 6	2/ 2	3/ 7	10/15
	None	2/ 2	—	0/ 2	2/ 4	1/ 2	—	1/ 2	2/ 4
	Subtotal	15/18(1)	1/12	2/18	18/48(1)	16/18	5/12	7/18	28/48
	Percent	83	8	11	37	89	42	39	58
NS	Right	3/ 5	0/ 5	1/19	4/29	3/ 5	0/ 5	1/19	4/29
	Left	6/ 9(2)	0/ 9	0/22	6/40(2)	6/ 9	2/ 9	3/22	11/40
	Bilateral	29/37(3)	4/18†	2/47(3)	35/102†(6)	31/36†	7/19	10/47	48/102†
	None	3/ 6	0/ 1	0/15	3/22	3/ 6	0/ 1	0/15	3/22
	Subtotal	41/57(5)	4/33†	3/103(3)	48/193†(8)	43/56†	9/34	14/103	66/193†
	Percent	72	12	3	25	77	27	14	34

*LAG = lymphangiogram interpretation: + = positive for para-aortic node abnormality; ±? = equivocal; − = negative.

†Para-aortic node biopsy or splenectomy omitted at laparotomy; laparotomy was not performed in 13 cases, and lymphangiograms in one additional case (NS histology). Three cases classified as lymphocytic depletion type and one unclassifiable case have been omitted from this tabulation.

still too few to warrant a firm recommendation that laparotomy be omitted in this group.

Finally, as noted previously (*1, 11*), the data of table 9 attest again to the striking association of liver and bone marrow involvement with concomitant (or prior) involvement of the spleen and para-aortic nodes. Of the 10 cases with biopsy-proved hepatic involvement, 9 were submitted to laparotomy; all had positive spleens, and 8 had positive para-aortic node biopsies. Similarly, although only 6 of the 9 patients with biopsy-proved bone-marrow disease were laparotomized, all 6 had both spleen and para-aortic node involvement. In addition to the 9 cases of concurrent spleen and liver involvement observed in this study, subsequent involvement of the liver after intervals ranging from 2–69 months was observed during serial follow-up examinations to date in more than 30 of our patients who initially presented with positive spleens and negative livers (as judged by liver function tests, liver scans, needle biopsies, and/or open biopsies). Johnson (*17*) argued that radiotherapy to the para-aortic nodes and spleen in patients with Hodgkin's disease of the NS type and negative lymphangiograms obviates the need for laparotomy and splenectomy. He based his view in part on the fact that he had never observed subsequent spread of disease to the liver in cases thus treated. However, although it is true that most of our cases with delayed hepatic spread were of the MC or LD variety, some were of the NS variety, and all had been irradiated to the para-aortic nodes and spleen (or splenic pedicle after splenectomy). Moreover, the data of table 4 clearly indicate that the hazard of occult involvement of the spleen in NS is by no means trivial; it was noted in 13% of cases with negative and 26% of those with equivocal lymphangiograms.

TABLE 9.—*Patterns of association with liver and bone marrow involvement**

	Total No. of cases	Number with laparotomy	Spleen positive	Para-aortic nodes positive
Liver biopsy positive	10	9	9	8
Bone-marrow biopsy positive	9	6	6	6

*All cases with positive liver or bone-marrow biopsy among 285 consecutive, previously untreated patients are represented. Of 3 patients with bone marrow involvement who did not have staging laparotomies, 2 had lymphangiograms, both of which were positive for para-aortic node involvement.

Apparently, the spleen is the critical site from which dissemination to either the liver or the bone marrow, or both of these prognostically very unfavorable Stage IV sites, is most likely to occur. It is this implication of spleen involvement that has led us to undertake "prophylactic" supplementary treatment of Stage III_S-A and III_S-B cases, after completion of total lymphoid radiotherapy, with either radioactive gold (^{198}Au) colloid (*13, 14*) or quadruple combination chemotherapy (*14, 18*); we have obtained highly encouraging results to date. Until new diagnostic methods are developed which can reliably detect small ($\leqq$1 cm diameter) foci of involvement in the spleen, laparotomy with splenectomy will remain the only way to establish the presence or absence of such involvement, and thus to formulate an optimal therapeutic regimen for each patient with Hodgkin's disease. Except for the specific subgroups mentioned, the yield of diagnostic information provided by staging laparotomy was consistently high, and as our surgical colleagues have reported (*19*), the observed complication rate, in the hands of experienced surgeons, is well within acceptable limits for an essentially diagnostic procedure.

Thus, from the data provided by this large series of consecutive, unselected, previously untreated patients, we feel justified in reaffirming that staging laparotomy is an important part of the diagnostic evaluation of all patients with Hodgkin's disease, except: 1) those with preoperatively apparent Stage IV disease (Ann Arbor classification); 2) those in whom advanced age or life-threatening medical disability contraindicates such surgery; 3) those with LP histology and negative lymphangiograms; 4) those with mediastinal adenopathy (alone or associated with other intrathoracic disease) in whom no cervical or supraclavicular adenopathy can be detected on very careful examination; and 5) possibly other subgroups yet to be defined as additional data become available—patients with Stage I involvement confined to the axillary nodes unilaterally may prove to be the first such example.

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Invited Discussion: Value of Staging Laparotomy With Splenectomy[1]

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THE PIONEER work carried out by the group at Stanford University School of Medicine on diagnostic laparotomy with splenectomy has certainly contributed much to the study of the natural history of Hodgkin's disease and to a more precise indication for the outline of a therapeutic program. This diagnostic procedure is now being performed in various centers.

At the National Cancer Institute of Milan, laparotomy was started in September 1970; as of March 1972, 185 patients with Hodgkin's and nonHodgkin's lymphomas have been splenectomized as part of our diagnostic approach to these diseases. I will briefly review the experience gained in the evaluation of the first 97 consecutive patients with Hodgkin's disease.

Exploratory laparotomy was not carried out in patients >68 years old (4 patients), in those with mild cardiovascular disturbances (3 patients), or in those who were markedly obese (3 patients). It was performed in 11 children whose average age was 8.8 years. Of the 97 patients considered, 26 were pretreated. Most of them were treated elsewhere with limited-field irradiation and the initial diagnostic work-up was incomplete.

Table 1 summarizes the results of diagnostic laparotomy in untreated and pretreated groups of patients. In untreated patients when, after all clinical and radiologic examinations were done, the disease appeared confined to lymph-node areas above the diaphragm (clinical Stage I–II), diagnostic laparotomy detected occult lesions below the diaphragm in 10 of 48 patients (20%). In particular, 9 patients had splenic involvement, and 1 patient had histologically positive nodes around the celiac axis. Of 3 patients with disease clinically below the diaphragm, none had occult intra-abdominal and, in particular, splenic involvement. In Hodgkin's disease with lymph nodes extending above and below the diaphragm (clinical Stage III), splenic involvement accounted for most of the occult lesions (total 50%) followed by celiac nodes and liver. Hodgkin's disease was clinically benign in 10 of 13 (77%) of the histologically positive spleens. Liver was found either palpable or classified suspicious when scanned in 2 of 4 patients in whom histology showed involvement by lymphoma. In 38% of pretreated patients who had an incomplete initial clinical staging performed elsewhere or suspicious signs of relapse after irradiation, diagnostic laparotomy detected occult abdominal lesions. In this group of patients, the percent of liver involvement was twice as high as that of untreated patients. As already reported by the Stanford group (*1, 2*), no patients had liver involvement without concomitant splenic involvement. While all patients with palpable spleen or positive scan had foci of Hodgkin's disease on histologic examination, liver was actually positive for lymphoma in only 10% of patients with either a palpable liver or a positive scan. Liver function tests were unreliable. Although lesions were focal, in no patients was microscopic evidence of splenic or hepatic involvement seen without grossly visible nodes. All 47 patients in whom a bone marrow biopsy was performed had no evidence of marrow infiltration. The results observed in Hodgkin's disease were similar to those found in the other types of lymphomas (*3*).

The spleen was positive in 6 of 23 patients (26%) with lymphocytic predominance (LP), in 7 of 24 (29%) with nodular sclerosis (NS), in 9 of 28 (32%) with mixed cellularity (MC), and

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Table I.—*Results of diagnostic laparotomy in 97 patients with Hodgkin's disease*

	Clinical extent before surgery	Number	Laparotomy		Occult abdominal involvement					Total patients
			Splenic involvement	Hepatic involvement	Retroperitoneal nodes	Celiac nodes	Mesenteric nodes	Spleen	Liver	
Untreated patients	Above diaphragm	48	9	1	2	2		9	1	10 (20%)
	Below diaphragm	3	0	0						0
	Above and below diaphragm	20	13	3		3	1	10	3	10 (50%)
	Total	71	22 (31%)	4 (6%)	2	5	1	19	4	20 (28%)
Pretreated patients	Above diaphragm	9	1	0				1		1
	Below diaphragm	5	2	0		2	1			2
	Above and below diaphragm	7	3	1		1	1	3	1	4
	Systemic symptoms ± suspicious involvement of spleen and/or liver	5	3	2		2			1	3
	Total	26	9 (35%)	3 (12%)		5	2	4	2	10 (38%)

in 9 of 21 (40%) with lymphocytic depletion (LD) types (table 2). In our series of patients splenic involvement was, therefore, more frequently associated with the unfavorable prognostic histologic expressions. In 6 patients (1 with MC, 3 with NS, and 2 with LD types), the spleen was involved apparently without retroperitoneal and hilar adenopathy. Of 6 patients with hepatic lymphoma, 5 had the LD-type tumor. The presence of systemic symptoms was also related to the presence of Hodgkin's disease in the spleen. In fact, 19 of 31 patients (61%) with splenic involvement had constitutional symptoms (table 2).

Table 2.—*Splenic involvement related to histologic type and to the presence of systemic symptoms*

Histologic type (Initial biopsy)	Patients	Splenic involvement With systemic symptoms	Splenic involvement Without systemic symptoms
LP	23	3	3
NS	24	4	3
MC	28	7	2
LD	21	5	4
Unclassified	1	—	—
Total	97	19(61%)	12(39%)

Complications related to laparotomy were minimal. There was no operative mortality. In about half of the patients a mild asymptomatic left pleural effusion was detected on chest X-ray 2–4 days after surgery. It spontaneously cleared within a few days. No severe infections were observed following splenectomy in adults and children even after myelosuppression during subsequent intensive chemotherapy and/or radiotherapy. In 4 patients (4%) a clinical and radiologic picture of intestinal occlusion 4–12 weeks after splenectomy was observed and a second laparotomy was required. From one to several adhesions among the intestinal loops were observed.

The incidence of abdominal disease, as detected after diagnostic laparotomy, was also related to the sites of cervical adenopathy (table 3). Though admittedly our numbers of patients are still too small to draw conclusions, we did not observe that patients with right unilateral cervical adenopathy showed a lower incidence of abdominal lesions (50% in our series) compared to those with left unilateral (50%) or bilateral (42%) cervical involvement. These data contrast with those reported by Dorfman (*2*) and by Kaplan and Rosenberg (*4*) who described a distinct relationship between the incidence of left cervical adenopathy and abdominal Hodgkin's disease. On the contrary, the presence of right unilateral cervical adenopathy (39%) was more frequently associated with mediastinal involvement than left unilateral cervical adenopathy (23%). Positive mediastinum was associated with bilateral cervical adenopathy in 39% of patients.

Table 3.—*Incidence of abdominal disease (with or without splenic involvement) related to site of cervical adenopathy*

Initial lymph node involvement	Patients	Abdominal involvement	(%)
Left cervical	31	15	(50)
Right cervical	24	12	(50)
Bilateral cervical	26	11	(42)
Mediastinal without cervical	7	5	(71)
Axillary without cervical and mediastinal	6	2	(33)
	94*	45	

*Three patients with disease limited to below diaphragm are excluded.

The effects of splenectomy on chemotherapy were examined in 52 patients subjected to intensive multiple drug combination with mechlorethamine, vincristine, adriamycin, bleomycin, and prednisone (*5*). In splenectomized patients treated with 6 cycles, the mean nadir of leukocytes (3,000/mm^3) and of platelets (170,000/mm^3) was higher compared to the mean nadir for those not splenectomized (1,900/mm^3 for leukocytes and 139,000/mm^3 for platelets). Therefore, asplenic patients could receive more myelosuppressive therapy. The 2 groups of patients were not well balanced as far as prior therapy was concerned. The comparison carried out among pretreated patients showed again that in splenectomized patients a higher total dose of myelosuppressive drugs could be administered. This difference was not observed in patients given only 2 cycles of quintuple chemotherapy.

In conclusion, exploratory laparotomy with splenectomy proved in our experience to be a useful diagnostic procedure for patients with Hodgkin's disease. It could reveal occult abdominal lesions, especially in those patients with clinical Stage III. In our series, the spleen was more likely to be involved in patients with certain histologic types of Hodgkin's disease and with systemic symptoms. Since all patients with hepatic involvement also had Hodgkin's disease in the spleen, a certain number of patients with histologically positive spleen may already have had occult foci of lymphoma in the liver. This is the main reason that, in our treatment program (*5*), chemotherapy precedes radiotherapy in Stage III patients (A and B). Exploratory laparotomy also allows the detection of abnormal nodes in abdominal lymphoid areas not covered by lymphography. However, since surgeons do not perform a systematic lymph-node dissection in these patients, a negative node sample with negative lymphography does not necessarily mean absence of disease in some surrounding nodes. Nevertheless, in our experience, lymphography proved to be a highly reliable diagnostic method. The false-positive findings were 1%; the false-negative findings 2%.

Evidence is growing that splenectomy increases tolerance to chemotherapy (*6*) and radiotherapy (*7*). Splenectomy allowed us to carry out an intensive course of chemotherapy with less leukopenia and thrombocytopenia occurring in the patient than was observed in patients receiving the same treatment with an intact spleen. As pointed out by Pannettiere et al. (*8*), asplenic patients received more myelosuppressive therapy per unit time because the shift of the blood cells into the circulating pool resulted in an exaggerated estimation of their total pool size. If splenectomy enhanced the tolerance to combination chemotherapy, it did not enhance the bone marrow reserve.

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Invited Discussion: Laparotomy Alters Treatment in Hodgkin's Disease [1,2]

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THE VALUE of high-dose, "radical," megavoltage, radiation therapy for early stage Hodgkin's disease has been well demonstrated (*1–6*). Radiation to major neck and trunk nodal areas [total-nodal (TN) irradiation] is currently under investigation for widespread nodal disease and thus far appears to control disease in a significant percentage of patients (*7, 8*). Patients with disseminated disease beyond the lymphoid tissue [Stage IV (*9*)] have benefited substantially from combination chemotherapy, with remissions sometimes lasting >4 years (*10*). The treatment philosophy for Hodgkin's disease in our center is based on the evidence for orderly progression in the spread of the disease in the great majority of cases (*11*). Stage I and II patients are treated with megavoltage irradiation to all *involved* lymph-node-bearing areas as well as to the next *adjacent* area. For example, left cervical and mediastinal disease would be treated by a "mantle" field followed by therapy to the para-aortic area. For this purpose, the spleen is considered to be a lymph-node-bearing area. Stage III-A disease is given TN irradiation. Treatment of patients with Stages III-B and IV is individualized, with most patients receiving combination chemotherapy. More recently, certain of these patients have been selected for combined therapy with TN irradiation and chemotherapy.

Accurate knowledge of the extent of disease is critical in deciding between radiation therapy or chemotherapy. Also, if irradiation is chosen, what nodal areas should be treated? More accurate and more detailed information about the extent of disease in the abdomen has been afforded by staging laparotomy and splenectomy than by all diagnostic maneuvers short of exploration (*12–18*).

In a previous report from this center (*19*), the influence of laparotomy on the management decision was studied in 54 unselected patients. Based on the therapeutic philosophy outlined, the treatment plan was altered in 20 of the 54 patients because of the procedure. The present paper expands this study to include an additional 60 patients who have had laparotomy and splenectomy.

MATERIALS AND METHODS

From April 1, 1969, to January 15, 1972, 181 patients with Hodgkin's disease were seen in consultation by the Joint Center for Radiation Therapy. One hundred and fourteen patients underwent laparotomy with splenectomy, wedge liver biopsy, and biopsies of intra-abdominal nodes.

Of the 67 patients who did not have laparotomy, 48 had disseminated Stage IV disease either proved by biopsy, or with strong clinical evidence of disease beyond nodes. Therefore, 133 patients were eligible for laparotomy. Of these eligible patients, 19 did not undergo the procedure for the following reasons: medical contraindications, in 7 patients; patient refusal, in 3; physician resistance, in 4; and extensive

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prior therapy, in 5. The 114 laparotomized patients (67 males and 47 females) ranged in age from 6 to 73 years (mean 30.1 yr); 23 had previous treatment from 4 months to 27 years before laparotomy.

Details of the routine preoperative evaluation and the surgical procedure were described in the previous report (*19*). A bone-marrow biopsy was performed in 99 patients. A lower extremity lymphangiogram was done in 105 patients; of the other 9, 4 were attempted but unsuccessful; the initial diagnosis of Hodgkin's disease was made at laparotomy in 2 patients; other studies indicated disease below the diaphragm in 2; and 1 patient was diagnosed as having Hodgkin's disease directly post partum.

Splenectomy was not done in 3 patients; liver biopsies were not taken in 4 patients; and retroperitoneal nodes were either not found or appeared normal and were not biopsied in 12 patients. In patients with positive or equivocal lymphangiograms, following retroperitoneal node biopsy, a postoperative film of the abdomen was obtained for confirmation of the biopsied nodes. Of 38 women <35 years old, 20 had an oophoropexy (*20*).

Preoperative clinical stage was compared to surgical staging in the 114 patients. The therapy that would have been given if laparotomy had not been done was compared with the actual treatment administered.

RESULTS

Alteration in Stage

In table 1, the stage of disease preoperatively is compared with that following laparotomy. As a result of laparotomy, 33 of the 114 patients (29%) changed stage, 18 (16%) dropped to a lower stage, and 15 (13%) advanced in stage. Of 91 previously untreated patients, 31 (34%) changed stage, whereas only 2 of 23 patients who had received prior therapy changed stage (9%). Patients staged II-B preoperatively were most prone to change (7/10). The presence or absence of systemic symptoms did not significantly affect the overall alteration in stage.

TABLE 1.—*Preoperative stage compared to stage after laparotomy in 114 Hodgkin's disease patients*

Stage before laparotomy	Stage after laparotomy							
	I	II-A	II-B	III-A	III-B	IV-A	IV-B	Total
I	9							9
II-A	1	18(3)		5(1)				24
II-B			3		5		2	10
III-A	3(1)*	10		25(5)				38
III-B			2		10(5)		3	15
IV-A		1		1		4(1)		6
IV-B							12(7)	12
Total	13	29	5	31	15	4	17	114

*Number in parentheses = No. of patients in each group who had received prior therapy.

Laparotomy was done in 18 Stage IV patients either because a previously treated single focus of extranodal disease, usually pulmonary,[5] was the sole reason for the Stage IV classification or because of uncertainty in the staging which warranted exploration.

Influence on Therapy

The treatment plan was altered by the findings at laparotomy in 40 of the 114 patients (35%), as shown in table 2. Planned pelvic

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[5] These patients would now be considered II_E or III_E according to the recent Ann Arbor Conference staging committee (*21*).

TABLE 2.—*Influence of laparotomy on treatment in Hodgkin's disease*

Number of patients	Alteration in therapy because of laparotomy						
	Less radiation	More radiation	Radiation instead of chemotherapy	Chemotherapy instead of radiation	Combined therapy instead of radiation alone	Chemotherapy instead of antimicrobial therapy	Number of patients changed therapy
77, systemic symptoms absent (A)	14	5	1	0	2	0	22 (28.6)*
37, systemic symptoms present (B)	4	5	1	4	2	2	18 (48.7)
Total 114	18	10	2	4	4	2	40 (35.1)

*Numbers in parentheses = percentage.

irradiation was not given to 18 patients with suspected abdominal nodes because disease was not found at laparotomy despite either a positive (11 patients) or an equivocal (7 patients) lymphangiogram. Ten patients had pelvic irradiation added because of unsuspected involvement of the spleen.

Two patients, considered Stage IV, were given nodal irradiation instead of chemotherapy after liver biopsies at laparotomy were negative. Liver involvement was proved at laparotomy in 4 patients who had been staged either II-B or III-B preoperatively, and these patients were treated with chemotherapy rather than radiation. Chemotherapy was added to nodal irradiation in 4 patients because of positive (1 patient) or suspicious (3 patients) infiltrates in the liver.

In two patients, Hodgkin's disease appeared to be disseminated, including probable liver involvement. However, the possible presence of intra-abdominal infection led to laparotomy. The liver biopsies showed Hodgkin's disease, and chemotherapy was instituted.

Accuracy of Diagnostic Procedures

Table 3 outlines the accuracy of clinical evaluation of the lymph nodes, spleen, and liver as determined by the findings at laparotomy. In general, there were more false positives in Stage A patients and more false negatives in Stage B patients. Twelve patients who had positive lymphangiograms were not included in table 3 because, in 4, normal-appearing nodes at surgery were not biopsied and, in 8, the biopsied negative nodes were not the nodes of interest on the films.

Table 3.—*Accuracy of clinical evaluation of disease in the abdomen compared to the findings at laparotomy in 114 patients with Hodgkin's disease*

	False positive	False negative	Number equivocal
Lymphangiogram	12/37	2/40	16/105
Spleen	5/24	30/90	—
Liver	5/9	3/85	20/114

Microscopic granulomas were seen in 13 patients: in the spleen in 9, in the liver in 7, in lymph nodes in 2, and in the bone marrow in 1. These patients are similar to those reported by Kadin et al. (*22*).

Mortality and Morbidity

One patient died 6 months after laparotomy because of a subdiaphragmatic abscess in the area of the previous splenectomy, resulting in a 0.9% mortality. Six patients were hospitalized for >14 days post laparotomy (17–42 days) because of a subdiaphragmatic abscess in 2, a wound abscess in 2, prostatic obstruction in 1, and axillary thrombophlebitis in 1.

DISCUSSION

The results of these studies support the conclusions of Kaplan and his co-workers concerning the usefulness of laparotomy in the staging of Hodgkin's disease. Previous reports (*12–18*) have indicated that staging was changed in 25–50% of patients because of laparotomy. However, no emphasis was placed on subsequent alteration of therapy, which is the ultimate criterion of the value of a diagnostic procedure. Further, the therapy decision can be altered by the findings at exploration although the stage is unchanged.

Using our treatment philosophy, the findings at laparotomy changed the treatment plan in 35% of the patients (40/114). In each center caring for Hodgkin's disease patients, the extent to which staging laparotomy and splenectomy influence the therapeutic decision depends on the treatment philosophy of that institution. Clearly, if TN irradiation is to be given to all patients, there would be less alteration of therapy as the result of the laparotomy findings. Conversely, in a center that treats only involved areas of disease, the influence of laparotomy on therapy would be greater than in our series.

The role of laparotomy must be continually re-evaluated. There may be subgroups of patients with specific histologic types in particular sites in whom the likelihood of intra-abdominal involvement is so small as to negate the value of laparotomy. However, we feel laparotomy is of importance in therapy decisions as long as our current therapeutic philosophy pertains and data on such subgroups are not yet complete. Hopefully, the therapeutic alterations brought about by the findings at laparotomy and splenectomy will improve survival and cure rates in Hodgkin's disease, since all diagnostic procedures influencing therapeutic maneuvers must ultimately be judged by the efficacies of these treatments.

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SUMMARY OF INFORMAL DISCUSSION ON STAGING LAPAROTOMY

S. A. Rosenberg commented that, in the earlier data from Stanford University, plus the more recent data from Yale University, there had been 28 patients with lymphocyte predominance or nodular sclerosis, and 1 or 2 clinical sites of involvement above the diaphragm, in 7 of whom (25%) the laparotomy had been positive. **H. Host** cited the Norwegian experience to date, which failed to support the observations from Stanford University concerning right versus left cervical node involvement and positive laparotomy. Among 75 laparotomies, there were 4 with right cervical adenopathy alone, all of which were positive, and 9 with left cervical adenopathy alone, only 3 of which were positive. **A. M. Jelliffe** stated that the laparotomy data of the British cooperative study also fail to reveal a significant left versus right preponderance. In patients with clinical Stage I-B or II-B disease, laparotomy had led to an increase in stage in 10 of the first 20 cases, with the spleen involved in all 10, the para-aortic nodes in 5, and the liver in 3.

S. B. Strum had gathered laparotomy data on 351 patients from several institutions (excluding Stanford University) and commented that they confirmed Doctor Kaplan's data on the absence of positive laparotomy findings in Stage I disease of certain sites. There had been 9 cases confined to the mediastinum, 10 in the upper right cervical, 6 in the upper left cervical, 4 in the axilla only, 6 submandibular, and 3 in the left or right inguinal region only, all of which had had negative laparotomies.

Value of Laboratory Indicators in Hodgkin's Disease: Preliminary Results [1,2]

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SUMMARY—The erythrocyte sedimentation rate (ESR), serum copper, ceruloplasmin, leukocyte alkaline phosphatase (LAP), muramidase, magnesium, and iron were measured in 112 consecutive, untreated patients with Hodgkin's disease and 75 concurrent controls. The ESR and serum copper showed the best correlation with disease activity, being elevated in 87% and 80% of patients, respectively. The serum iron and LAP appeared of less value, being abnormal in 57% and 24% of patients, respectively, while the serum muramidase and magnesium did not correlate with disease activity. Preliminary evidence suggested that the ESR and serum copper should be considered as complementary examinations which may be of value in monitoring a patient's response to treatment and possibly in detecting extension or recurrence of disease. However, further analysis of serial values obtained over an extended period of time is needed before their exact roles can be established.—Natl Cancer Inst Monogr 36: 315–323, 1973.

HODGKIN'S DISEASE often behaves erratically; its manifestations may be subtle, and recurrence or extension of disease may occur 1–5 years or more after apparently effective therapy. Attempts to assess the activity of Hodgkin's disease have relied on routine history and physical examination, radiologic investigations, and certain laboratory tests. However, these have often proved inadequate as a monitor of the effectiveness of a specific treatment regimen and in the early detection of recurrence and/or extension of disease. Renewed interest in the possible usefulness of certain laboratory tests as indicators of disease activity has been stimulated by reports that these tests yield abnormal values in patients with active disease and return to normal during periods of disease inactivity. Included among such tests have been the serum copper and ceruloplasmin, total white blood cell count, absolute lymphocyte count, erythrocyte sedimentation rate (ESR), and leukocyte alkaline phosphatase (LAP) which are elevated and the serum iron and zinc which are depressed.

We chose to establish a longitudinal, prospective study obtaining pretreatment and serial post-treatment laboratory tests in an attempt to establish a useful index of disease activity and to evaluate the potential of these tests as prognostic indicators. The tests obtained were as follows: ESR, serum copper and ceruloplasmin, LAP, serum muramidase, magnesium, and iron. This communication presents our preliminary evaluation of these tests based on an analysis of

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the pretreatment and immediate post-treatment laboratory values in a series of 100 cases.

MATERIALS

Serum and anticoagulated blood were obtained from 112 consecutive, untreated patients with biopsy-proved Hodgkin's disease who were part of a controlled, randomized, clinical trial of the treatment of Hodgkin's disease by radical radiotherapeutic techniques and/or chemotherapy. Patients were excluded from the analysis if the initial laboratory tests were obtained when the patient was taking estrogen-containing medications or when there was evidence of an acute inflammatory condition. There were 12 such patients, leaving 100 to form the basis for this report. The serum iron was only recently added to the investigation; therefore, results were available for analyses in only 30 patients. The patients' ages ranged from 18 to 58 years with a median of 24 years. There were 60 males and 40 females. The patients were staged according to the recent Ann Arbor classification (*1*) after an extensive diagnostic evaluation including "staging" celiotomy (*2*). There were 16 patients with Stage I disease, 35 with Stage II, 32 with Stage III, and 17 with Stage IV disease. Their distribution according to histology is shown in table 1.

TABLE 1.—*Distribution of patients according to histology*

Histologic types	Number of patients
Nodular sclerosis	82
Mixed cellularity	16
Lymphocyte predominance	2
Lymphocyte depletion	0

METHODS

All tests were performed by the Clinical Laboratory, Stanford University School of Medicine, except for the ceruloplasmin determination which was performed by Bio-Science Laboratories, Van Nuys, California. Anticoagulated blood and serum were collected, and laboratory tests were performed by the same technician, who had no prior knowledge of the patient's diagnosis or clinical status. Duplicate determinations were performed for each sample tested for copper, magnesium, and the ESR. Only single determinations were obtained for the muramidase, LAP, ceruloplasmin, serum iron, and total iron-binding capacity (TIBC). Quality control was maintained by use of standardized, commercially available control sera. Normal values for all tests except the serum iron and TIBC were determined from 75 concurrent sex- and age-matched healthy controls obtained incidental to blood bank donations. The mean ±2 standard deviations was taken as defining the normal range (table 2). The normal range for the serum iron and TIBC was provided by the Clinical Laboratory.

To provide continuing quality control, a stock control serum obtained from a normal male was divided into 5-cc samples and frozen; duplicate samples were run with each group of patient samples.

ESR.—The Wintrobe sedimentation method was used. Values were corrected for the presence of anemia. Whole anticoagulated blood was allowed to settle in a 120-mm long tube at room temperature, and the amount of settling was recorded at 1 hour. Normal values: males, 0–10 mm; females, 0–20 mm.

Serum copper.—The serum copper was determined by atomic absorption spectroscopy by a modification of the method of Olson and Hamlin (*3*). Normal values: males, 63–139 μg/100 ml; females, 80–144 μg/100 ml.

Ceruloplasmin.—Ceruloplasmin was determined from the rate of oxidation of phenylenediamine at 37° C and at a pH of 6.0. The rate of appearance of the purple oxidation product was measured spectrophotometrically. Normal values: 282–570 U.

Magnesium.—Magnesium was determined by atomic absorption spectroscopy. Normal values: 1.6–2.4 meq/liter.

Serum iron.—The serum iron was determined by the method of Caraway (*4*). Normal values: males, 80–160 μg/100 ml; females, 60–135 μg/100 ml. TIBC: 250–350 μg/100 ml.

LAP.—The method of Rutenberg et al. (*5*) was used to determine the LAP activity of neutrophilic leukocytes. Normal values: 0–160 U.

Muramidase.—The serum muramidase was determined by a modification of Litwack's method (*6*). The muramidase index was also calculated based on a differential leukocyte count obtained the same day. Normal values: muramidase, 12–40 μg/ml; index, 3–12 μg/ml.

OBSERVATIONS

The range, mean, and standard deviations of the various laboratory tests for the control and study groups are presented in table 2. There was a highly significant difference between the mean values of the ESR, copper, and ceruloplasmin tests for the study group and those of controls. The frequency of abnormal and normal pretreatment values for 5 of the 6 tests is presented in table 3. The frequencies with which abnormal values were obtained with the ceruloplasmin, copper, and ESR tests did not differ significantly ($P>0.05$) from each other. Multiple regression analyses revealed no significant relationship between the frequency of abnormal values and sex, age, histology, and/or specific sites of involvement.

TABLE 2.—*Range, mean, and standard deviations of the various laboratory tests for the control and study groups*

Laboratory test	Control group		Study group		Statistical significance
	Range	Mean ± SD	Range	Mean ± SD	
ESR mm/hr					
Males	1–12	4 ± 3	4–62	30 ± 12	$P<0.01$
Females	0–26	10 ± 5	4–64	37 ± 14	$P<0.01$
Copper μg/ml					
Males	56–143	101 ± 19	89–266	172 ± 43	$P<0.01$
Females	80–148	112 ± 16	79–330	192 ± 64	$P<0.01$
Ceruloplasmin units	240–591	426 ± 72	130–1240	791 ± 252	$P<0.01$
LAP units	0–160	80 ± 40	3–216	101 ± 59	$P<0.01$
Muramidase, μg/ml	4–46	26 ± 7	2–60	25 ± 12	$P>0.05$
Magnesium, meq/liter	1.4–2.5	2.0 ± 0.18	1.5–2.3	1.9 ± 2.0	$P>0.05$

TABLE 3.—*Frequency of abnormal and normal pretreatment values in 100 patients*

Test	Normal No. of patients	Abnormal No. of patients
ESR	13	87
Ceruloplasmin	20	80
Cu^{++}	20	80
LAP	76	24
Muramidase	96	4
Magnesium	98	2

Table 4 is an analysis of 4 of the tests according to stage and presence or absence of systemic symptoms. A significant relationship ($P<0.05$), as judged according to a test of Order in Proportions (7), existed between the frequency of abnormal values in all 4 tests and the extent of disease. Although analysis of the entire patient sample revealed no significant relationship ($P>0.05$) between these tests and the presence or absence of systemic symptoms, isolation and analysis of Stage II patients alone revealed that those patients with B symptoms had a much greater frequency of abnormal ESR, copper, and ceruloplasmin values than those without systemic symptoms. Although there are statistical reservations regarding the isolation and analysis of one cohort of patients from an entire sample, this finding in Stage II patients remains intriguing.

The serum iron and TIBC values for 30 patients were available for analysis. The mean serum-iron level for 12 females was 84 μg/100

TABLE 4.—*Correlation between the frequency of normal and abnormal pretreatment ESR, ceruloplasmin-copper, and LAP values and stage and presence or absence of systemic symptoms**

Stage	Number of patients	ESR		Ceruloplasmin-Cu^{++}		LAP	
		Normal	Abnormal ↑	Normal	Abnormal ↑	Normal	Abnormal
I-A	15	6	9	6	9	14	1
I-B	1	0	1	0	1	1	0
Total	16	6 (37)	10 (63)	6 (37)	10 (63)	15 (94)	1 (6)
II-A	20	2	18	9	11	16	4
II-B	15	2	13	0	15	13	2
Total	35	4 (11)	31 (89)	9 (26)	26 (74)	29 (83)	6 (17)
III-A	16	3	13	3	13	10	6
III-B	16	0	16	2	14	14	2
Total	32	3 (9)	29 (91)	5 (16)	27 (84)	24 (75)	8 (25)
IV-A	7	0	7	0	7	3	4
IV-B	10	0	10	0	10	5	5
Total	17	0	17 (100)	0	17 (100)	8 (47)	9 (53)
Grand total	100	13 (13)	87 (87)	20 (20)	80 (80)	76 (76)	24 (24)

*Numbers in parentheses are percentages.

ml with a standard deviation of 47, and for 18 males it was 103 μg/100 ml with a standard deviation of 59. The correlation between the serum iron, TIBC, hematocrit, and stage of disease is shown in table 5. Hypoferremia was seen in 17 of 30 patients (57%), whereas only 3 patients showed a decrease in the TIBC and 4 were anemic. There was a significant correlation ($P<0.05$) between progression of stage and incidence of hypoferremia. In 2 patients with hypoferremia and hematocrits of <34%, the anemia was not characteristic of iron deficiency and their bone marrows revealed adequate iron stores. The serum iron could not be restored to normal with oral or parental iron supplementation.

TABLE 5.—*Correlation between the frequency of normal and abnormal pretreatment serum iron, TIBC, and hematocrit (HCT) values and stages of disease in 30 patients**

Stage	Number of patients	Serum iron		TIBC		HCT	
		Normal	↓	Normal	↓ Abnormal	Normal	<34%
I	5	4	1	5	0	5	0
II	10	6	4	10	0	9	1
III	10	2	8	8	2	9	1
IV	5	1	4	4	1	3	2
Total	30	13(43)	17(57)	27(90)	3(10)	26(87)	4(13)

*Numbers in parentheses are percentages.

Neither the serum magnesium nor muramidase appeared to correlate with disease activity. Magnesium values obtained during or within 1 month of completing treatment were not elevated as has been described by Ilicin (*8*).

In general, the ceruloplasmin and copper values paralleled each other. Statistical analysis supported the findings of Warren et al. (*9*) that the increase in total serum copper was due to an increase in ceruloplasmin concentration and that the serum non-ceruloplasmin copper remained relatively constant. Care must be taken in interpreting isolated ceruloplasmin and copper values, since elevated values were obtained in 50% of patients with an overt bacterial infection and in 100% of patients taking estrogen containing oral contraceptives. This is in accordance with the findings of other investigators (*10*).

Although we ultimately hope to establish the value of these tests in the long-term management of patients with Hodgkin's disease, sufficient time has not elapsed to allow definitive analysis. Two anecdotal cases below show certain characteristic patterns that are emerging in patients followed with serial values.

Case 1 (text-fig. 1): W.D., a 20-year-old white male with Stage IV-B nodular-sclerosing Hodgkin's disease, had an abnormal ceruloplasmin, copper, and ESR before therapy. After extended-field radiotherapy plus colloidal radioactive gold, all values slowly decreased to the normal range where they have remained. During this period the patient was without evidence of disease.

We have also seen other patterns in this clinical setting. In 30 of 100 cases analyzed, the ceruloplasmin, copper, and ESR values actually rose immediately after completion of therapy and remained so for months, only then to show a slow decline to normal values over a period of 3–4 months. In approximately 20% of patients followed for 1 year or longer after completion of treatment and considered to have no evidence of disease, the ESR remained abnormally elevated but relatively stable, whereas the ceruloplasmin and copper values returned to normal. The ultimate fate of these patients will have to be analyzed to determine the usefulness of these tests.

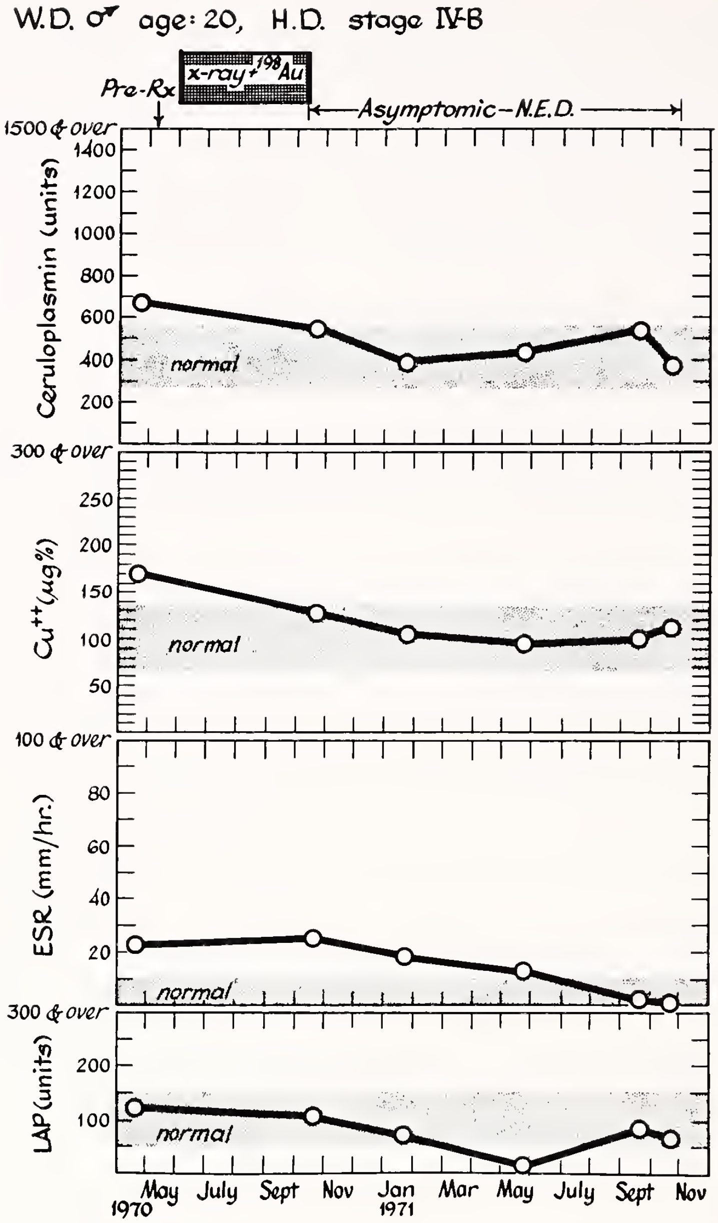
Case 2 (text-fig. 2): J.M., a 65-year-old Mexican-American male with Stage IV-B nodular-sclerosing Hodgkin's disease, had abnormal values for all 4 tests previous to initiation of therapy. After completion of 6 cycles of combination chemotherapy, all values remained elevated except the LAP. The serum copper and ceruloplasmin rose steadily during a period in which the patient was asymptomatic but had palpable evidence of continued disease activity. The ESR, while remaining elevated, did not accurately reflect the progression of disease. Due to increasing symptoms, the patient was begun on chlorambucil in May after which the ceruloplasmin and copper values decreased whereas the ESR remained unchanged and the LAP increased.

DISCUSSION

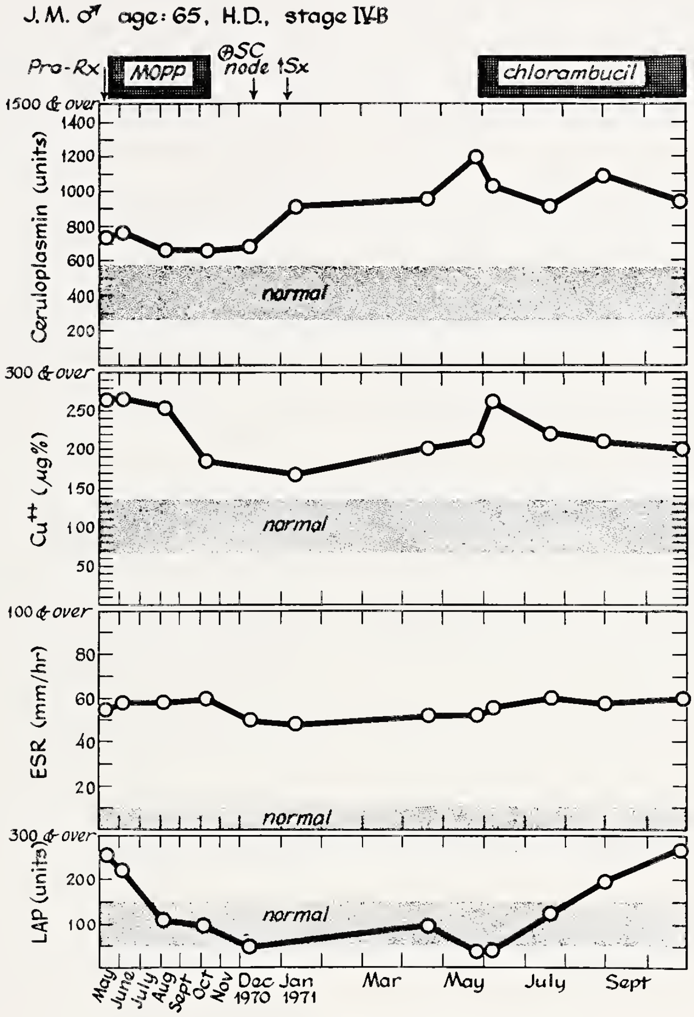
The long-term purpose of this study is to determine the value of certain laboratory tests for predicting or establishing the clinical status of patients with Hodgkin's disease and to see if they have prognostic significance. Although limited follow-up precludes definitive conclusions at this time, certain trends can be discerned from our analysis of the pretreatment and immediate post-treatment values.

Of those tests performed, the three most reliable indicators of disease activity appeared to be the ESR and the serum ceruloplasmin and copper. We consider the sedimentation rate and serum copper as being complementary to each other, since in 50% of cases in which one test did not accurately reflect the patient's status, the other did. The ESR has the advantage of being both simple and inexpensive to perform. A statistically significant correlation existed between the frequency of abnormal values and the stage of disease. The fact that these tests were abnormal in only 63% of Stage I cases may infer that their ability to detect early relapse is limited.

Although the mean values of the ESR, copper, and ceruloplasmin tests for the study group were significantly different from those of the control group (table 2), the practical usefulness of these tests as diagnostic adjuncts was lessened by the extreme patient variability. The degree of variability is shown by the large standard deviation associated with each of these tests in the study group. Because of this variability, we found it extremely important in patients followed with serial examinations to analyze the values according to the trend in each patient rather than considering just single, isolated



Text-figure 1.—Sequential laboratory values in a case of Hodgkin's disease illustrating the relation between laboratory indicators and clinical course in a 20-year-old male with Stage IV-B nodular-sclerosing Hodgkin's disease.



TEXT-FIGURE 2.—Correlation between laboratory indicators and clinical course in a 65-year-old male with Stage IV-B nodular-sclerosing Hodgkin's disease.

values. Thus, although 20% of patients with abnormal pretreatment values also had elevated values after completion of treatment, most of the latter group actually had shown a significant decrease as compared to their pretreatment values. Some of these patients' subsequent values returned to normal and some remained elevated but stable, whereas others became progressively elevated and were associated with recurrent disease.

Our experience with the serum copper and ceruloplasmin tests was in accord with the findings of others (*11–13*). The preliminary results infer that serial determinations may be valuable in the assessment of a patient's response to treatment and/or detecting relapse or recurrence. Rising values in the absence of infection or estrogen therapy may suggest occult active Hodgkin's disease. Since a linear relationship existed between the ceruloplasmin and copper values, there appeared to be no reason to perform both. We have chosen to eliminate the ceruloplasmin determinations, since the copper assay by atomic absorption is inherently more accurate than the test for ceruloplasmin which relies on an enzymatic process.

We cannot confirm or deny the conclusion of Teillet et al. (*14*) that "biological signs" are important in the detection of splenic relapses, since all but one of our patients with splenic involvement also had supradiaphragmatic and/or para-aortic node involvement. Although these investigators did show an apparent correlation between abnormal laboratory indicators and splenic relapse, they did not report the extent of disease at the time of splenic relapse. Therefore, we cannot be sure that the abnormalities they observed in their laboratory tests were not just a reflection of the extensiveness of disease rather than a specific indicator of splenic involvement.

Cline and Berlin (*15*) reported that hypoferremia, which has been attributed to a defective reutilization of iron (*16*) in patients with Hodgkin's disease, was one of the first signs of active disease. In our experience the serum iron level did not appear to be an extremely sensitive indicator of disease activity, since only 17 of 30 patients (57%) demonstrated hypoferremia. The hypoferremia did not appear to be due to transferrin deficiency, since the TIBC was normal in 90% of patients. This is in accordance with the findings of Jaffe et al. (*17*) in pediatric patients with Hodgkin's disease, but contrasts with Cline's data which showed a low serum iron-binding capacity. Although a positive correlation was established between the stage of disease and frequency of abnormal values, we cannot confirm the observation of others (*14*) that hypoferremia is the best indicator of the systemic syndrome. This may be due partially to our small sample size.

Under our conditions, the LAP was only elevated in 24% of patients with active disease. This finding does not support the work of others (*18–20*) who have shown a 65–95% positive correlation between abnormal LAP values and disease activity. Jaffe et al. (*17*) also noted a similar discrepancy in their data. The exact cause is uncertain. The patients in these other series were not as well staged as ours and may have included a greater percentage of patients with disease of incompletely appreciated extent who had a greater probability of having abnormal laboratory results. The best correlation between disease activity and LAP was reported by Bennett et al. (*20*), who, unfortunately, did not analyze their patients according to stage. Since this test is only semiquantitative and somewhat subjective, subtle differences in the preparation of the slide or the method of scoring may also have been a contributing factor.

Published reports (*21, 22*) offer inconclusive evidence as to the relation between serum or erythrocyte magnesium and disease activity in Hodgkin's disease. Some investigators reported elevated magnesium levels whereas others showed the levels to be decreased or unchanged. Our data support the latter observation. We could not confirm the presence of an elevated serum magnesium during or following treatment, even in patients with large tumor volumes treated aggressively. Although the attainment of cardiotoxic levels of magnesium following rapid destruction of large tumor masses may be a potential problem, as pointed out by Ilicin (*8*), it must occur rarely under our conditions.

The two major limitations to the use of even the most promising of these laboratory tests as reliable diagnostic adjuncts are their relative nonspecificity and the degree of variability exhibited by the study group. Although the ESR and serum copper show potential value as monitors of disease activity, their true value must await the availability of firm data regarding serial results and their fluctutations in patients in remission and relapse. Because of the paucity of such data, we believe that a progressive elevation of the ESR or serum copper in the absence of other obvious causes should prompt diagnostic studies to determine if occult disease is present. However, it should not form the basis for a therapeutic decision in a patient with quiescent Hodgkin's disease unless there is other firm documentation of disease activity.

CONCLUSIONS

1) The ESR and serum ceruloplasmin and copper showed the best correlation with disease activity. The frequency of abnormal values showed a statistically significant relationship with advancing stage of disease.

2) No significant relationship could be established between frequency of abnormal laboratory values and sex, age, histology, systemic symptoms, and/or specific sites of involvement.

3) The LAP and serum iron appeared to be of limited value under our conditions, and the serum muramidase and magnesium did not correlate with disease activity.

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Invited Discussion: Value of Fluctuations in the Serum Copper Level in the Control of Patients With Hodgkin's Disease[1,2]

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AT THE Middlesex Hospital, the serum copper concentration is determined by high-resolution flame spectrophotometry (*1*). This method is quick, sensitive, and extremely accurate. The repetition of estimations on specimens kept for up to several years has constantly produced results within 1–2% of the original level. No chemical method is as efficient. Our normal variation ranges from 82 to 148 μg/100 ml, and we regard a level of greater than 150 μg/100 ml as definitely raised.

As already indicated by Ray et al. (*2*) this is not a specific test for Hodgkin's disease. Many other diseases have been reported to be associated with a raised serum copper. Hepatic cirrhosis, many acute and chronic infections, collagen disorders, cardiac infarction, lymphomas other than Hodgkin's disease, leukemias, and other neoplasms arising in many parts of the body are associated with a raised serum copper. In the normal, generally fit individual, the copper level rises after surgery, with the administration of estrogens and contraceptive pills, and during pregnancy. But although nonspecific, it is one of the most delicate indications of active Hodgkin's disease, particularly when carried out regularly over a long period.

In our original publication from the Middlesex Hospital (*3*), after >5,000 serum copper estimations in 600 patients, we analyzed the findings in 64 subjects with Hodgkin's disease who had been followed for 3–45 months. Early in our investigation we found a linear correlation between the copper level and the plasma ceruloplasmin concentration. This made this second, more complicated investigation unnecessary as a routine procedure. In this publication, we found that most patients with Hodgkin's disease who responded well to radiotherapy or chemotherapy showed a rapid fall in the serum copper level. Sometimes there was an initial rise of short duration, but this was followed, typically, by a rapid fall to below the normal maximum level of 150 μg/100 ml within 5–6 weeks. A few patients had a slow fall of serum copper over many months, the period of time extending in 1 case to 1 year before the normal level was reached. Satisfactory control of this disease is therefore indicated not by the speed of the fall but by the downward direction of the serum copper level, and the rapidity of the fall is unrelated to the final outcome. Eighteen patients showing a fall in copper with control of the disease were reported in our original publication in 1969. All 18 have remained under control up to the present time, 3 years later, confirming our original optimistic views. Of these 18 patients, 3 did not have a copper level >150 μg/100 ml before treatment commenced, but control of the disease was followed by a fall to levels lower than those found initially. Presumably these patients had a normal serum copper in the very low range.

In the same publication, we referred to 2 other groups. The first group, containing 27 patients who died of active Hodgkin's disease, revealed a completely different picture. The serum copper level fluctuated wildly, and, as the

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] This study is supported by the Aitchison Charitable Trust, The Batchworth Trust, The Cancer Research Campaign, The J. S. Fraser Trust Fund, the New Court Charitable Trust, the Peggy Russell Memorial Fund, and Eli Lilly & Co., Ltd.

disease progressed, there was a tendency for the level to rise. The level rose to nearly 400 μg/100 ml in many patients, and the highest level was 720 μg/100 ml. The pattern commonly seen was a fluctuating high level with a penultimate rise when the highest level for that individual was usually reached. Frequently this extremely high peak was followed by a terminal fall, which was often precipitate and sometimes dropped to the normal level. The second group contained 10 patients who were alive, but had active Hodgkin's disease that remained uncontrolled for periods of 8–40 months. There was a fluctuation in the copper level similar to, but not extreme as that seen in the previous group, and, in most, it remained constantly above the normal maximum range. However, 3 of these 10 patients had fluctuating low levels in the presence of active disease. Since this original publication, we have seen 2 more patients with active disease in whom the serum copper level has never risen above the normal maximum of 150 μg/100 ml. These may be examples of patients in whom the normal serum copper is extremely low, but because the disease has never been successfully controlled, the individual's normal level has never been demonstrated. A "normal" serum copper level, therefore, does not always indicate satisfactory control of Hodgkin's disease.

Recent alterations in the staging of patients with Hodgkin's disease by procedures such as a laparotomy have prevented any attempt to relate the long-term copper level fluctuations with the stage and degree of activity of the disease process. Possibly a statistically significant relationship may evolve in the next few years as more patients who have been laparotomized have serial serum copper estimations. At the present time, we can say only that our continued observations over the last 3 years confirm the views expressed in our publication in 1969. In general, the serum copper level estimated by high-resolution flame spectrophotometry is rapid, efficient, and accurate, and this investigation mirrors the control of Hodgkin's disease in nearly all patients. Control of disease is accompanied by a steadily falling copper level and lack of control by a fluctuating and usually high level. When control is followed by a relapse, this is usually accompanied by an increase of copper from a normal to a high level, and often this rise precedes any clinical sign of disease. This rise has been observed up to 6 months before disease was clinically or radiologically demonstrable. Clearly, the serum copper level is of no diagnostic use in Hodgkin's disease but, when used for long-term assessments, it is of considerable value.

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SUMMARY OF INFORMAL DISCUSSION ON LABORATORY INDICATORS

M. Tubiana discussed another problem in assessing the prognostic value of laboratory indicators carried out at initial examination. It is well known that most of the laboratory indicators and especially the erythrocyte sedimentation rate (ESR) are correlated with the presence or absence of systemic symptoms. That makes it difficult to distinguish between the influence of systemic symptoms and that of laboratory indicators on prognosis.

In the European cooperative clinical trial data, the presence or absence of systemic symptoms and the ESR value were strongly correlated. Among the patients with a low ESR value, the presence or absence of systemic symptoms had no influence on the proportion of patients in remission. Analysis of actuarial survival of 4 subgroups of patients, all in clinical Stage I or II, revealed that among the groups with or without systemic symptoms with low ESR the survival was about the same, but there was a significantly poorer survival in the group with a high ESR and systemic symptoms. The proportion of patients in remission of those surviving at 4 years is about the same in the subgroups without systemic symptoms or with systemic symptoms and low ESR, but in the subgroups with systemic symptoms and high ESR the prognosis is much poorer.

In a previous study of patients treated between 1922 and 1966, a high ESR was of prognostic value only for patients without systemic symptoms; there was no influence of ESR for patients with systemic symptoms. In the recent data, however, for patients treated with more extensive radiotherapy and with higher doses, there is no influence of ESR among patients without systemic symptoms and, on the other hand, there is an influence of ESR among patients with systemic symptoms. The simplest interpretation is that, with the type of therapy previously available, the treatment was efficient only for patients without systemic symptoms and with low ESR, and that now, with a much more effective treatment, the prognosis has improved for all the subgroups, and it is only in the worst group with symptoms that the high ESR is still of some prognostic value.

The patients in the European cooperative clinical trial were divided into 2 subgroups. One was treated only with radiotherapy and revealed a very significant correlation between the ESR value and the proportion of recurrences. The other half of the patients were treated by combination radiotherapy and chemotherapy, which for these patients was a more efficient type of treatment; in these patients, there was no significant correlation between the value of ESR and the proportion of recurrences. In conclusion, we see that the value of any laboratory indicator is very strongly correlated with the type of treatment. Of course, when we reach a treatment so efficient that all patients are cured, there will be no prognostic value to any type of laboratory indicator.

G. H. Fairley commented that the important point is that abnormal values of any laboratory indicator should never be taken as an indication for treatment, but only as an indication to search for relapsing disease.

P. Wolf stated that the nitroblue tetrazolium reduction test had been reported to be abnormal in patients with Hodgkin's disease. However, in about 20 patients studied to date at Stanford University, the test had yielded generally low scores and no correlation with disease activity.

Panel Discussion

The Ann Arbor Staging Classification

Chairman: Paul P. Carbone

INTRODUCTION

The panel has been asked to discuss clinical experiences and problems encountered to date in using the clinical staging classification which we proposed at Ann Arbor.[1] The principal difference from the earlier Rye classification was that localized extranodal disease is no longer classed as Stage IV but denoted with the suffix E. Also the classification of Stage II now includes 2 or more lymph-node regions on the same side of the diaphragm and the subscript S was put in to designate spleen involvement. Disseminated disease was defined as involvement of the bone marrow, the liver, or multiple extralymphatic sites. The other change was in symptoms. New evidence was presented that significant weight loss (>10%) is prognostically unfavorable, whereas itching was not correlated with prognosis, so weight loss was substituted for pruritus as a "B"-symptom.

I would also like to present some recent data obtained by T. A. Waldmann of the National Cancer Institute in a study of IgE levels in patients with various malignancies and diseases. Doctor Waldmann noted that there are only 2 malignant conditions associated with abnormalities, one of which was chronic lymphocytic leukemia with exceptionally low levels, and the other of which was Hodgkin's disease. The geometric mean level of untreated patients with Hodgkin's disease was approximately 13 times that of normal individuals and there was almost 100% correlation with the presence of B-symptoms. These are very new data. Obviously the question is: "What is the relationship of this to other prognostic factors?" Doctor Waldmann and others on our staff will be looking at this to see what happens with treatment.

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[1] CARBONE PP, KAPLAN HS, MUSSHOFF K, et al: Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 31:1860–1861, 1971.

Diagnostic Evaluation and Clinical Staging in Hodgkin's Disease: Usefulness and Problems of the Ann Arbor Staging Classification in Primary Staging and Staging in Relapse [1]

John E. Ultmann *and* **Edgar M. Moran,** *Department of Medicine, Section of Hematology, University of Chicago, Pritzker School of Medicine, and Division of Biological Sciences and the Argonne Cancer Research Hospital (Operated by the University of Chicago for the U.S. Atomic Energy Commission), Chicago, Illinois 60637*

SUMMARY—The advantages of the Ann Arbor modification of the Rye staging system of Hodgkin's disease are reviewed. An outline of the workup for the staging of Hodgkin's disease is presented. Additional procedures, including azygography, bone scanning, and bronchial brushing biopsies are discussed. Our experience in staging patients who have been staged and treated previously is summarized.—Natl Cancer Inst Monogr 36: 333–345, 1973.

ADOPTION of the Ann Arbor modification (*1*) of the Rye staging system (*2*) (*see* below) has resolved a number of problems encountered with the Rye system. The most important are: 1) Applicability of the new staging system to lymphoma as well as to Hodgkin's disease. 2) Consideration of an extralymphatic organ or site as a primary focus or as a direct extension from an adjacent lymphatic site (*1, 3*); these presentations are more favorable than was thought heretofore and may, in fact, be curable (*3*). 3) Separation of nodal from splenic disease, with emphasis on the prognostic significance of the latter in predicting hepatic involvement, particularly in Hodgkin's disease. 4) Emphasis on a dual system of stage designation according to Clinical Staging (CS) only and according to Pathologic Staging (PS); this permits comparison of *all* cases by clinical staging and comparison of biopsy-proved sites by pathologic staging where applicable.

To stage patients as accurately as possible, it is important to search out sites of involvement by appropriate techniques and to document histologically, when feasible, that such involvement is indeed Hodgkin's disease. The Committee on Hodgkin's Disease Staging Procedures of the Ann Arbor Conference has urged that certain procedures be used in staging patients, so that the CS and PS are more accurate, and thus their comparison between centers can be easily accomplished (*see* recommendations).

Castellino and Blank (*5*) reviewed the place of conventional radiographic procedures, including lymphangiography, in the diagnostic evaluation of patients both at presentation and in relapse of disease. Hoffer et al. (*6*) and Peckham (*7*) presented data on the usefulness of gallium-67 (^{67}Ga)-citrate scanning in patients with Hodgkin's disease.

In addition to the procedures already mentioned, special radiographic and radioisotope techniques may occasionally be useful in identifying sites of tumor involvement.

Azygography has been carried out by Dr. Klaus Ranniger, of our institution, in more than 100 patients with Hodgkin's disease or lymphoma. Among these, only a few cases of lymphoma demonstrated displacement of the azygos vein, indicating presumptive evidence of posterior mediastinal lymph-node enlargement. In

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

Hodgkin's Disease: Ann Arbor Modification of Rye Staging System (1971)

Stage I: Involvement of a single lymph node region (I) or of a single extralymphatic organ or site (I_E).

Stage II: Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of extralymphatic organ or site and of one or more lymph node regions on the same side of the diaphragm (II_E). Optional recommendation: Number of node regions involved indicated by subscript, e.g., $II_{(3)}$.

Stage III: Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by localized involvement of extralymphatic organ or site (III_E) or by involvement of the spleen (III_S), or both (III_{SE}).

Stage IV: Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node enlargement. The reason for classifying the patient as being in stage IV should be identified further by defining site by symbols.

In Hodgkin's disease, all patients are subclassified to indicate the absence (A) or presence (B) of: 1) unexplained body weight loss of $>10\%$; 2) unexplained fever with temperatures above 38° C; and 3) night sweats.

CS—Clinical Staging
PS—Pathologic Staging

Abbreviations for tissues sampled:

S—spleen; H—liver; N—lymph nodes (other than primary); M—marrow; L—lung; P—pleura; O—osseous; and D—skin.

Staging Classification Examples

CS I-A PS $I_{S-H-N-M-}$	Implies clinical Stage I without symptoms and pathologic Stage I; and negative spleen following splenectomy, liver biopsy negative, additional node biopsy negative, and marrow biopsy negative.
CS II-$A_{(3)}$ PS $III_{S+H-N+M-}$	Implies clinical Stage II-A, 3 node regions involved, PS III with spleen positive, abdominal lymph node positive, liver biopsy negative, and bone marrow biopsy negative.
CS III-B PS IV_{S-H+M-}	Implies clinical Stage III-B with PS IV due to positive liver biopsy; marrow and spleen are negative.
CS IV-B_{LH} PS IV_{H+M-}	Implies clinical Stage IV-B with gross evidence of lung and hepar involvement and PS IV due to positive liver biopsy; marrow biopsy is negative.

Recommendations on Staging Procedures

A. *Required Evaluation Procedures*
   1. Adequate surgical biopsy, reviewed by hematopathologist
   2. Detailed history (?fever, sweating, pruritus, and weight loss)
   3. Complete physical examination with attention to lymphadenopathy, Waldeyer's ring, liver, and spleen; and bone tenderness
   4. Laboratory studies:
      Complete blood count, platelet count, erythrocyte sedimentation rate, serum alkaline phosphatase level
      Evaluation of renal function and liver function
   5. Radiologic studies:
      a. Chest roentgenogram (posterior-anterior and lateral views)
      b. Intravenous pyelogram
      c. Bilateral lower extremity lymphangiogram
      d. Skeletal survey, especially thoraco-lumbar vertebrae, pelvis, proximal extremities, and areas of bone tenderness and/or pain

B. *Required Evaluation Procedures Under Certain Conditions*
   1. Whole-chest tomography, if abnormality on chest roentgenogram
   2. Inferior cavography for equivocal lymphogram or pyelogram
   3. Bone marrow biopsy, by a needle or open-surgical technique if
      a. Serum alkaline phosphatase elevated

    b. Unexplained anemia or other blood count depression
    c. Roentgenographic or scintigraphic evidence of osseous disease
    d. Generalized disease of Stage III category or greater
4. Exploratory laparotomy and splenectomy, if management decisions will depend on identification of abdominal disease

C. *Useful Ancillary Procedures not Definitive for Diagnosis*
    1. Skeletal scintigrams
    2. Hepatic and spleen scintigrams
    3. Serum chemical analyses, including calcium and uric acid
    4. Estimate of patient's delayed hypersensitivity

D. *Procedures and Tests Promising for Clinical Study at Selected Centers but Experimental at This Time*
    1. Whole-body gallium-67 and selenium-75 scintigrams
    2. Determinations of serum iron and iron-binding capacity, copper and ceruloplasmin, zinc, haptoglobin, fibrinogen, α_2-globulin; as well as urinary hydroxyproline, leukocyte alkaline phosphatase, absolute lymphocyte count, antibodies to Epstein-Barr virus, and human lymphocyte antibody typing

none of these patients were conventional radiologic studies positive. However, in none of the patients with Hodgkin's disease studied were the azygograms positive. The procedure cannot be recommended as routine in the workup of patients with lymphoreticular neoplasms.

Scanning of bone marrow with technetium-99m (^{99m}Tc) sulfur-colloid carried out during performance of liver-spleen scans may occasionally reveal involvement with Hodgkin's disease when radiologic evidence is lacking. Fluorine-18 bone scans may or may not be positive in such instances. Studies are currently in progress to correlate and compare these various techniques.

The problems encountered in diagnosing lung infiltrates stimulated development of bronchial brushing and bronchial forceps biopsies as diagnostic procedures. Dr. John Fennessy of our Radiology Service, in collaboration with Dr. Henry Rappaport and Dr. Daina Variakojis of our Laboratory of Surgical Pathology, are responsible for this project at the University of Chicago. A representative patient is a 16-year-old girl who presented in April 1969 at the University of Chicago Hospitals and Clinics with an 8-month history of pruritus, recent night sweats, and weight loss. Physical examination was unrevealing. Radiologic examination of the chest showed a mediastinal mass and a contiguous infiltration in the anterior segment of the right upper lobe (fig. 1). Bronchial brushing and bronchial forceps biopsy showed Hodgkin's disease, nodular sclerosis (NS) (figs. 2–4). A complete staging workup, including laparotomy and splenectomy, was negative. Consequently, a thoracotomy and a right upper- and right middle-lobectomy were performed. Final staging was Hodgkin's disease, NS CS II$_E$-B PS II$_{S-H-N-M-L^+}$. Postoperatively, the patient received megavoltage, extended mantle therapy to a tumor dose of 4000 rads (completed Aug. 1969). From April to November 1970, she received 6 cycles of combination chemotherapy with Cytoxan (cyclophosphamide), Oncovin (vincristine sulfate), procarbazine (Matulane), and prednisone (COPP). She is in complete remission at this time (Feb. 1973).

Since then, additional experience has been gained in utilizing the technique of bronchial brushing for diagnosis of abnormalities in patients suspected or proved to have lymphoreticular neoplasms or in patients with lymphoma suspected of having an opportunistic infection. The results obtained in these studies are summarized in table 1.

TABLE 1.—*Bronchial brushing**

	Number of patients	Pathologic finding	
		Bx-proved	Bx-negative
Hodgkin's disease	6	3	3
Lymphoma	3	1	2
Opportunistic infections			
Pneumocystis carinii	5	4	1
Varicella	1	1	0
Nocardia	1	1	0
Aspergillus	1	1	0

*Courtesy Dr. J. Fennessy, Dr. H. Rappaport, and Dr. D. Variakojis.

TABLE 2.—*Staging Hodgkin's disease after initial therapy**

Patient case No. age (yr)/ sex	Initial status			Evolution intercurrent events and therapy†	Interval between first staging and reassessment	Reassessment		
	CS before treatment	Therapy	Result			CS	PS	Therapy
1. 23/F	III-A MC	^{60}Co-MX 4500	CR chest	—	2 yr	III-A (abdomen not previously treated)	S–H+N+M–	VdG-TN 4000 + COPP
2. 23/F	II-A NS	^{60}Co-IF 3000	CR	RR p 3 yr ^{60}Co-MX 3000 RR p 5 yr VdG-IF 3000 LR p 6 yr ERT IF 4000	6 yr	II-A→RR→LR	S–H+N+M– E+ (chest wall)	ERT-IF 4000
3. 24/F	II-B NS	^{60}Co-IF 4500	PR	—	4 mo	II-B→LR	S+H–N–M–	VdG-EM 4000
4. 26/F	I-A NS	kv-IF 3000	CR	RR p 2 yr kv-IF 2000 LR p 5 yr kv-IF 2200	9 yr	I-A→RR→RR	S–H–N–M–	VdG-EM 4000
5. 24/F	III-A NS	VdG-TN 3000	CR	—	5 yr	III-A→Rec.	S+H–N+M–	COPP
6. 42/M	III-A MC	VdG-TN 3500	CR	—	5 yr	III-A→Rec.	S+H–N+M–	VdG-IF 4000
7. 20/F	I-A NS	^{60}Co-IF 4000	CR	RR p 1½ yr ^{60}Co-IF 3000 TDR p 3½ yr ^{60}Co-IF 3500	4 yr	I-A→TDR→Rec.	S+H–N+M–	VdG-TN 4000 + COPP
8. 24/F	II-A NS	kv-IF 2400 kv-IF 2000 ^{60}Co-IF 3800	CR	—	3 yr	II-A→RR	S–H–N–M–	VdG-MX 4000
9. 21/M	CS II-B NS PS II S-H-N-M-	^{60}Co-TN 3000	PR	—	1 yr	II-B RR$_E$	H–N–M– E+ (pectoral muscle)	VdG-MX 3200‡ + ERT 3800
10. 29/F	I-A NS	kv-IF 2300	CR	—	3 yr	I-A→LR & RR	S–H–N–M–	VdG-IF 4000
11. 49/F	II$_E$-A NS	^{60}Co-EF 2500–3350	CR	—	3 yr	II$_E$-A→LR & RR	S–H–N–M–	VdG-MX 4000 ERT-IF 1400

12. 26/M	II-A NS	kv-IF 550 ^{60}Co-IF 1000	PR	—	11 yr	II-A→TDR	S–H–N–M–	VdG-MX 4000
13. 16/M	I-A NS	VdG-MX 4000	CR	—	3 yr	I-A→TDR	S–H–N+M–	VdG-Y 4000
14. 23/M	I-A NS	^{60}Co-EF 3200	CR	—	2 yr	I-A→LR & TDR	S+H–N+M–	VdG-TN 4000 + ERT-IF 1200
15. 28/F	III$_S$-A NS	VdG-TN 4000	CR	RR p 1 yr VdG-IF 4000 LR p 4 yr VdG-IF 3500	4 yr	III$_S$-A→Rec.→EL	S–H–N+M– D+	VdG-IF 4000§ ERT-IF 4000
16. 24/F	CS III-A NS PS III S+H-N+M-	VdG-TN 4300	CR	—	3 yr	III$_S$-A→EL	H+N+M–	COPP
17. 26/F	I-A NS	kv-IF 3600	CR	TDR p 3 months & LR's; kv-IF 1000–3600	3 yr	I-A→TDR→EL	S+H–N+M–	VdG-TN 4000 + COPP
18. 48/F	II$_S$-B NS	^{60}Co-IF 4000	PR	—	6 mo	II$_S$-B→EL	S+H+N+M–	VLB + Pred.

*CS = Clinical Stage
PS = Pathologic Stage

MC = Mixed Cellularity
NS = Nodular Sclerosis

kv = 250 kv Radiotherapy
^{60}Co = Cobalt-60 Radiotherapy
VdG = Van de Graaff Generator Radiotherapy
ERT = Electron Beam Radiotherapy

IF = Involved Field
EF = Extended Field

MX = Maltese Cross
EM = Extended Mantle
Y = Inverted "Y"
TN = Total Nodal

CR = Complete Response
PR = Partial Response

Rec. = Recurrence
LR = Local Recurrence
RR = Regional Recurrence
RR$_E$ = Regional Recurrence with Extralymphatic Involvement
TDR = Transdiaphragmatic Recurrence
EL = Extralymphatic Recurrence

COPP = Cytoxan + Oncovin + Procarbazine + Prednisone, Cyclic treatment x 6
VLB = Velban

†The time of the intercurrent events is calculated from the initial therapy.
‡Patient refused completion of planned radiotherapy.
§Chemotherapy for EL not applicable because of documented inactive coccidioidomycosis.

A few comments regarding the staging of patients following initial therapy or following relapse are in order. The staging classification proposed in Ann Arbor (*1*) specifies that both the clinical and the pathologic staging classification apply *only* to the patient at the time of disease presentation and *prior* to definitive therapy. The only exception might be that pathologic staging by laparotomy may be done after a course of treatment when symptoms (e.g., mediastinal or spinal cord compression) demand that treatment to the upper half of the body must be given first. Obviously the burden of thoroughness in the evaluation of the patient lies heavily on the first team of physicians who stage the patient. Unfortunately, patients are often seen after errors have been made in clinical or pathologic staging or after errors in therapeutic management. Reassessment and determination of correct stage may be helpful in selected cases. In addition, a significant number of patients come to the physician in relapse and require careful evaluation to determine treatment strategy.

To adhere to the recommendations proposed at *this* meeting (*8*), we have used the following designations for patients in relapse: *local recurrence:* a recurrence in an area previously treated; *regional recurrence:* a recurrence outside the previously treated areas which, however, is confined to the same side of the diaphragm where disease was found initially; *transdiaphragmatic recurrence:* recurrence of disease in lymph nodes (or in spleen) but on the other side of the diaphragm than originally noted; and *extralymphatic recurrence:* appearance of disease in extranodal or extrasplenic sites.

During the past 3 years we have had the opportunity to stage 18 patients who have had previous clinical staging 4 months to 11 years before being seen by us. The initial status, including clinical stage, therapy, and result are shown in table 2. Two of the eighteen patients had pathologic staging 3 years (patient #16) and 1 year (patient #9) before the second staging procedure. In 5 patients (#2, 4, 7, 15, 17), intercurrent events led to recurrences and additional therapy was given (table 2).

The case of a 24-year-old woman (patient #16) seen for the first time at another medical center illustrates problems encountered in reassessment of patients in relapse. In August 1968, the patient presented with painless left supraclavicular lymphadenopathy. She had no symptoms. Biopsy showed Hodgkin's disease, NS. Clinical stage was CS III-A and pathologic stage, PS $\mathrm{III}_{\mathrm{S^+H^-N^+M^-}}$. The patient received total-nodal radiotherapy (Van de Graaff generator, 4300 rads tumor dose) and went into complete remission. In May 1969, she developed a nephrotic syndrome, which improved on steroid therapy. In June 1969, she had an episode of herpes zoster which healed rapidly. Beginning December 1970, she complained of fever and anorexia. Physical examination, routine blood tests, liver function tests, radiologic examinations (including chest film, gastrointestinal series, pyelogram, and lymphangiogram), and liver biopsy were negative. The patient was then transferred to our hospital for further workup. ^{99m}Tc liver-"spleen" scan showed a single filling defect in the liver and no spleen uptake; however, the ^{67}Ga-scan revealed a left upper quadrant mass (fig. 5). Repeat ^{67}Ga-scan confirmed this observation. In view of these findings, we believed that the patient had an extralymphatic recurrence. A second staging laparotomy was performed. This revealed massive peripancreatic lymph nodes in the position indicated by the ^{67}Ga-scan (fig. 6); liver biopsies showed Hodgkin's disease. The histologic pattern was NS. The patient was placed on cyclic combination chemotherapy with COPP and entered a complete remission in September 1971. She has been maintained in a complete remission on bis-chlorcethyl-nitrosourea (BCNU) at monthly intervals to the present time (Aug. 1972).

Among the patients (table 2), patient #1, originally staged CS III-A, was found to have been treated incompletely with a mantle only; our assessment was compatible with residual infradiaphragmatic disease in lymph nodes being present 2 years later. Pathologic staging revealed findings strongly suggestive of extralymphatic extension (S–H+N+M–). The patient was given total-nodal radiotherapy followed by 6-cycle chemotherapy with COPP. She has been free of disease 15 months. Two patients were thought to have true recurrences only: In patient #2, laparotomy was negative; in patient #3, an unsuspected transdiaphragmatic recurrence was found. Eight patients (#4, 5, 6, 7, 8, 9, 10, 11) were thought to have recurrences above and/or below the diaphragm: In each instance, the laparotomy findings supported the clinical impression. Three patients appeared to have developed transdiaphragmatic recurrences: in patient #12, this was not confirmed; but in patients #13 and 14, it was proved by laparotomy and splenectomy. Four patients were suspected of having extralymphatic extensions: In patients #15, 16, and 18, this was confirmed; in patient #17, it could not be proved.

In summary, of the 7 negative laparotomies, 1 resulted in a change in stage from TDR to RR and only a mantle treatment was given (patient #12). Of the 11 positive laparotomies, 3 revealed unsuspected findings: CS III→EL (patient #1), LR→TDR (patient #3), and EL→TDR (patient #17).

In all instances, therapeutic measures could be based on information obtained from the surgical staging. These are summarized for each patient in table 2. There was no postoperative morbidity or mortality in this group of patients. It is concluded that meticulous pathologic staging, including laparotomy and splenectomy when indicated, is useful and feasible in the workup of patients with Hodgkin's disease in relapse.

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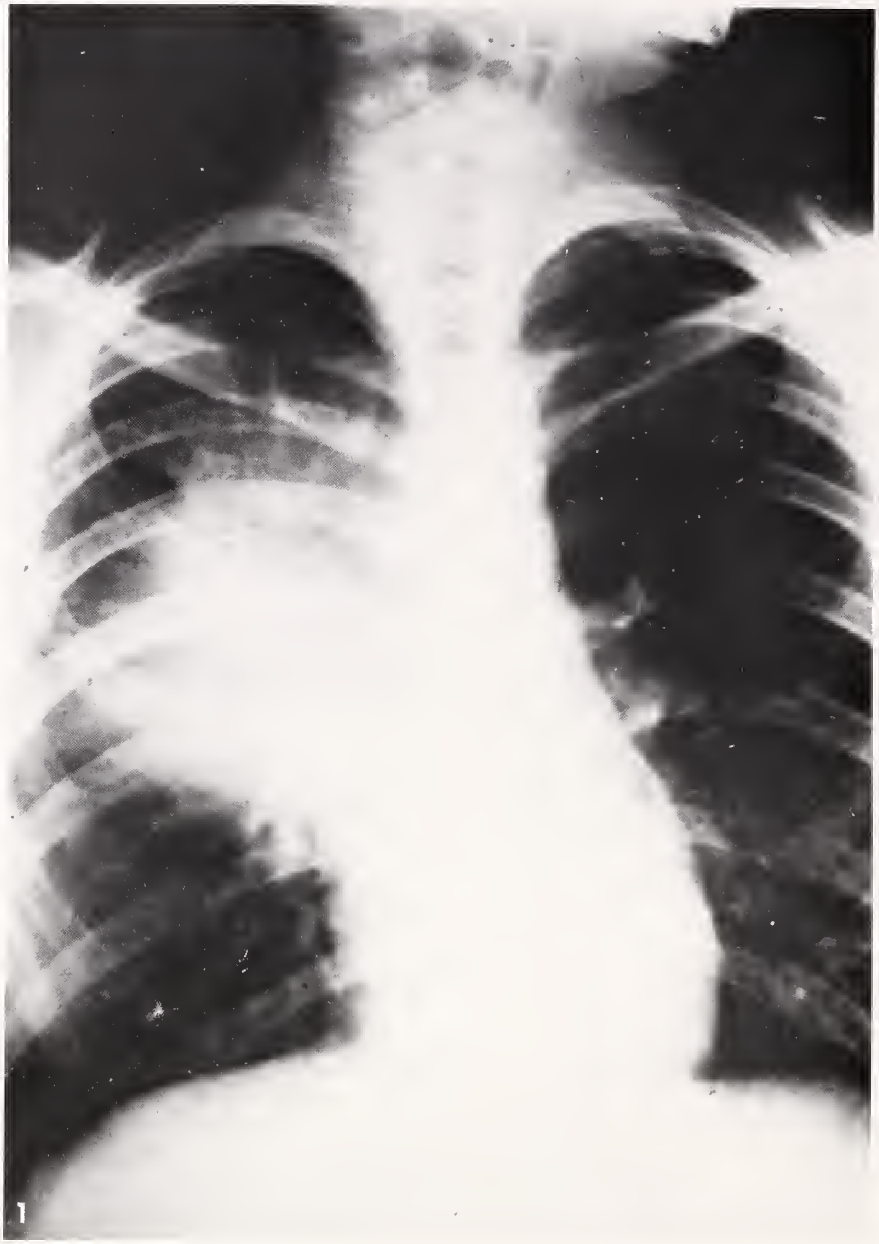

Figure 1.—X-ray of chest of 16-year-old girl with 8-month history of pruritus and recent night sweats and weight loss, showing mediastinal mass and contiguous infiltration in the anterior segment of the right upper lobe.

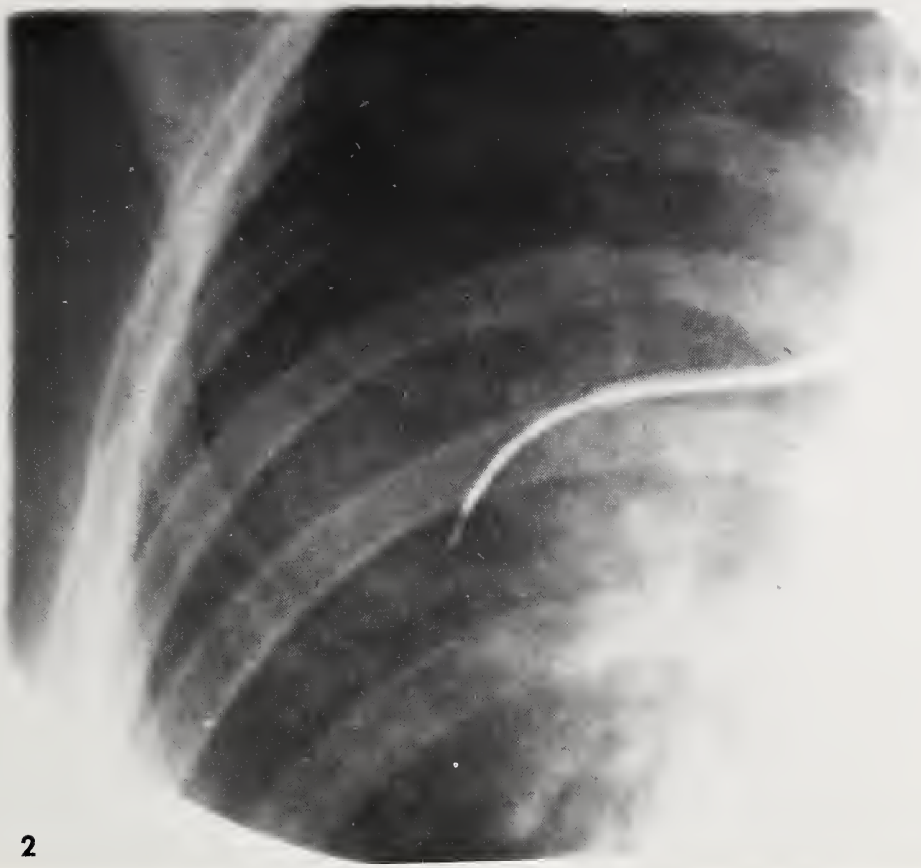

FIGURE 2.—Bronchial brushing of right upper lobe.

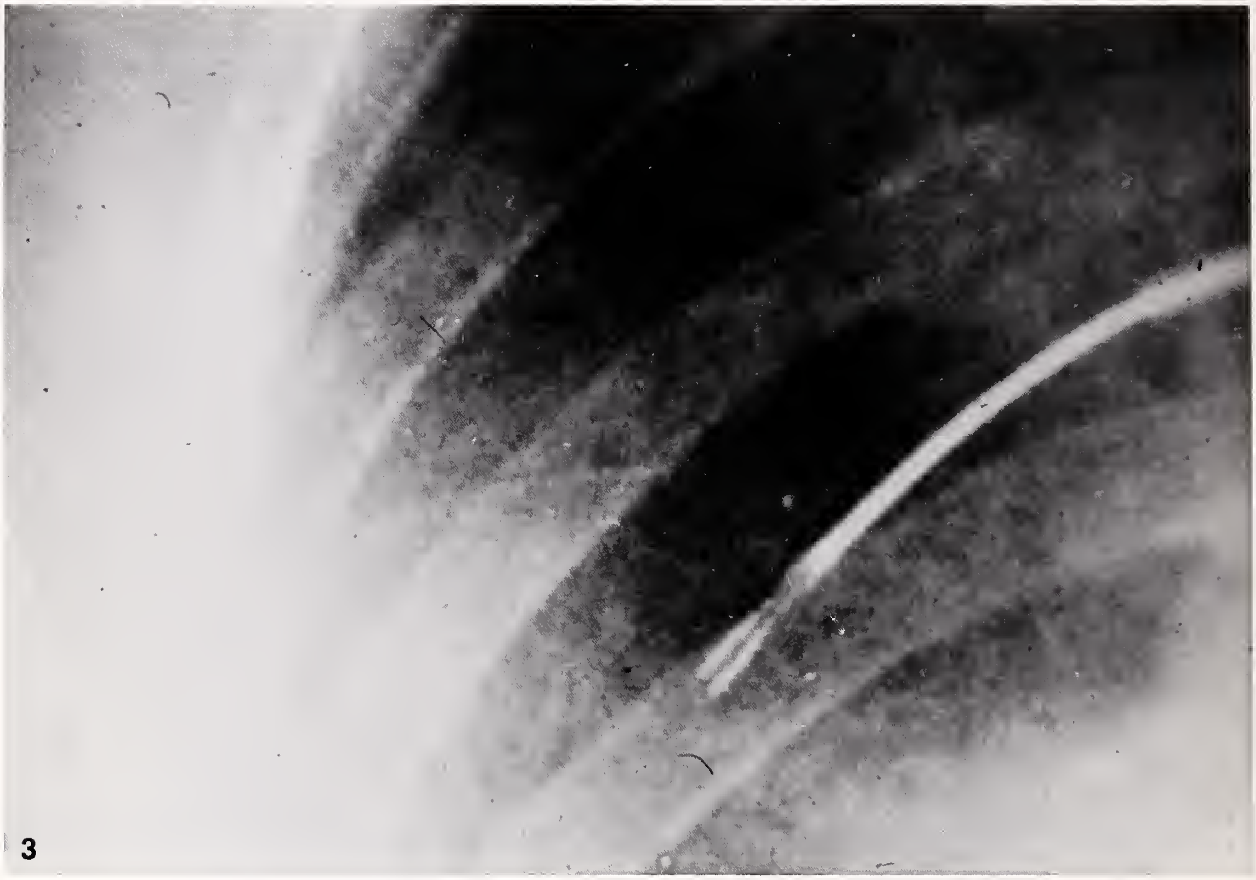

FIGURE 3.—Bronchial forceps biopsy of right upper lobe.

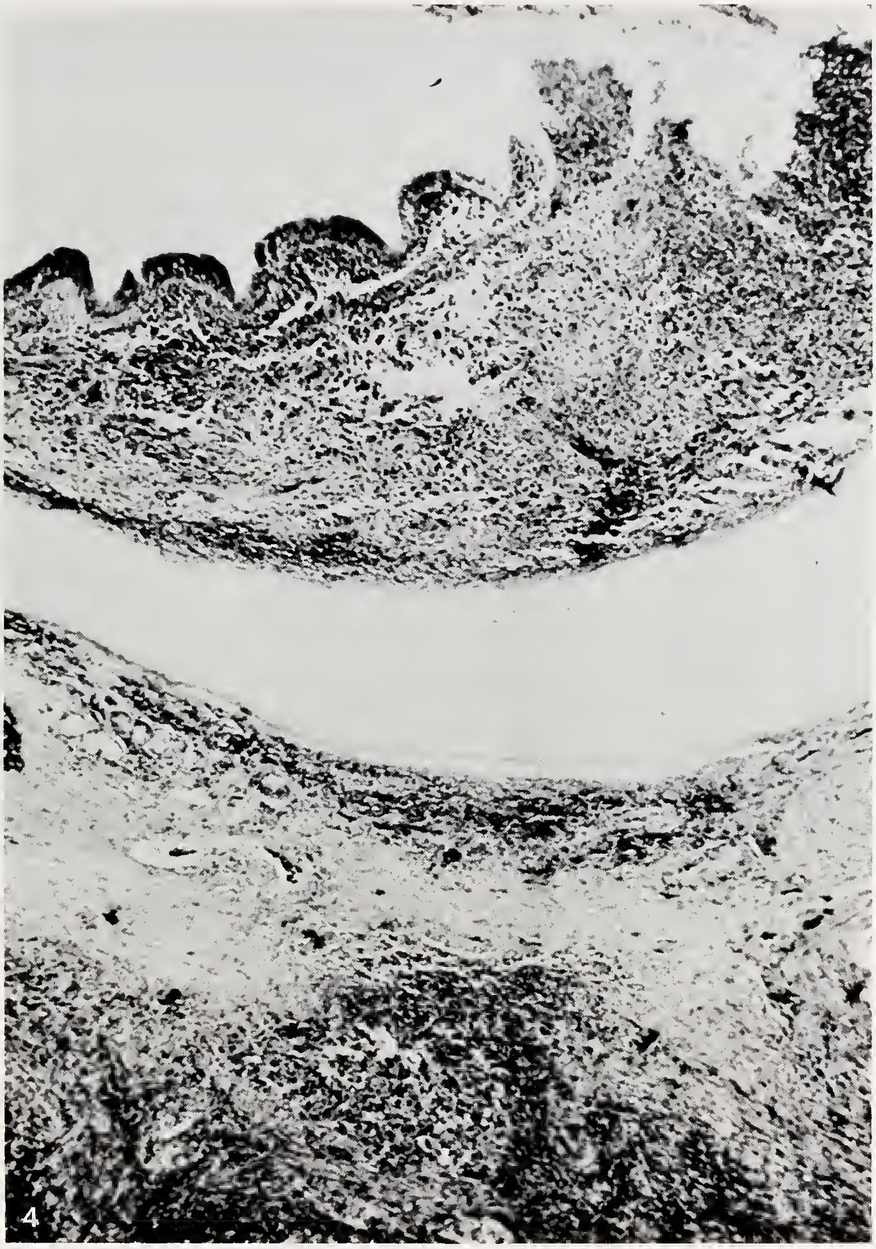

FIGURE 4.—Track of bronchial forceps catheter as seen in histologic section of tumor in lobectomy specimen. × 100

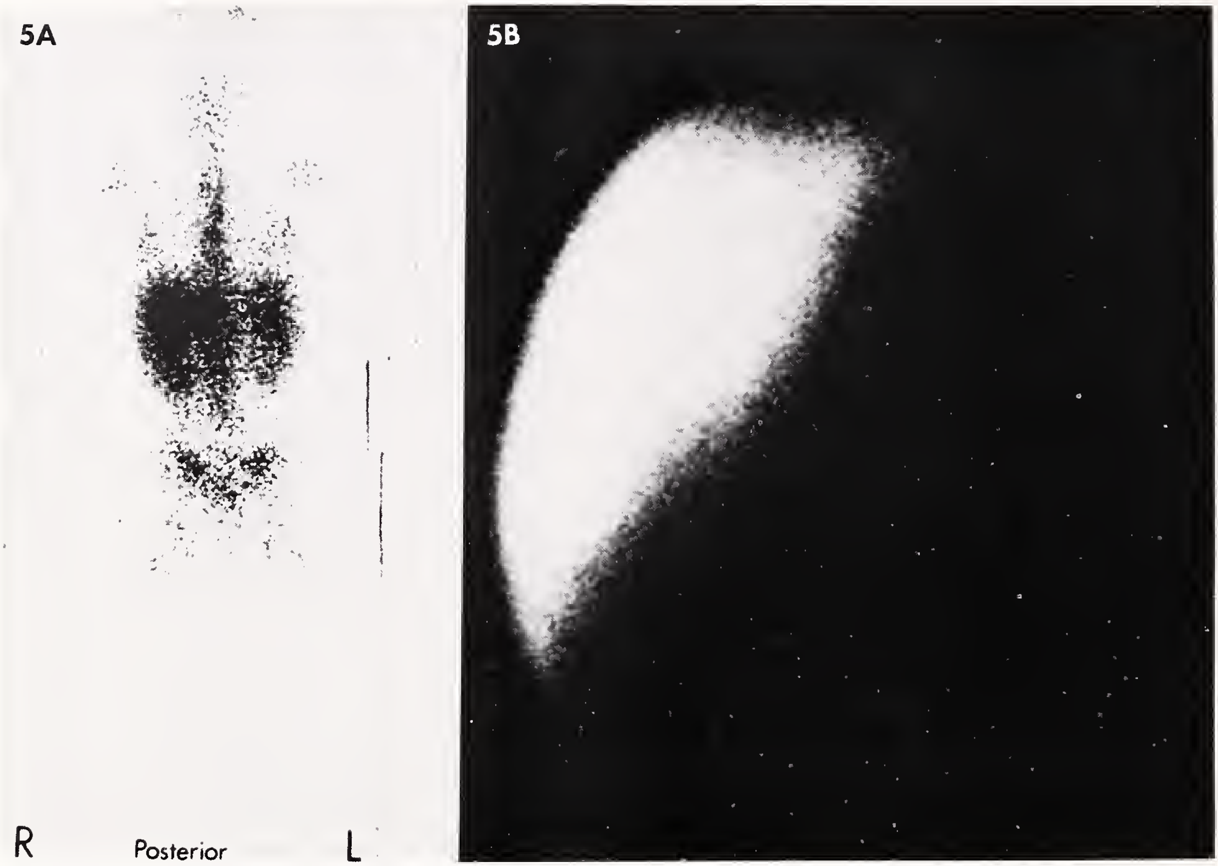
5A
5B
R
Posterior
L

FIGURE 5.—The ^{67}Ga-scan (*A*) reveals a left upper quadrant mass. The ^{99m}Tc liver-"spleen" scan (*B*) fails to show spleen.

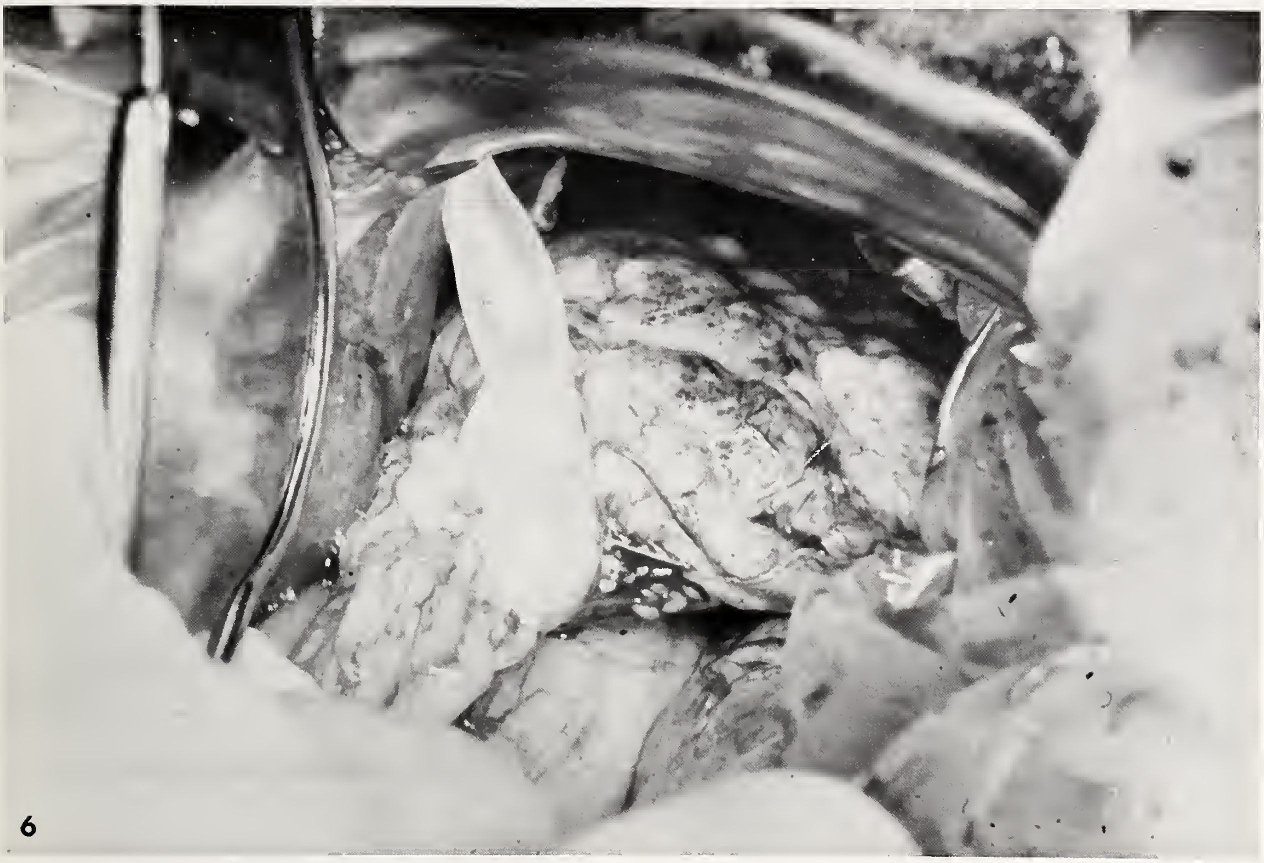

Figure 6.—In situ view of the operative field. The costal arch is shown cephalad. Massive peripancreatic lymph nodes are seen in the position indicated by the ^{67}Ga-scan.

SUMMARY OF INFORMAL DISCUSSION BY THE PANEL

S. A. Rosenberg commented that in his opinion the Ann Arbor staging classification does excellently the job that was asked of it—to provide comparability among major centers studying the disease. This classification also does the job of separating clinical stage from pathologic stage. But he fears the classification has become quite complicated. For those experts doing research in this field, there will be no problem in its use. But one has to be concerned about the much larger number of physicians who also see Hodgkin's disease patients and who may have difficulty in understanding the classification. He believed that the use of letter symbols to denote sites of Stage IV involvement, and of plus and minus symbols to indicate positivity or negativity, turns many physicians away from using the Ann Arbor classification. He thought it would probably be best not to use the subscripts, unless one were preparing the kind of detailed chart that Doctor Ultmann presented in which each patient individually could be seen and exactly what was done to the patient and how the staging changed.

M. Tubiana agreed with what Doctor Rosenberg had said. He thought that the Ann Arbor staging classification is extremely useful; but does it have to be as complicated as it looks? One can speak about pathologic Stage III or pathologic Stage II without giving all the subscripts. The subscripts are only useful when one wants to describe the patient in detail. Then the subscripts are extremely convenient because if one uses symbols one can describe both what has been done for the diagnostic evaluation of the patient and the findings.

J. E. Ultmann commented on one point of ambiguity in using the new Ann Arbor classification. When we refer to Stage III, we mean lymph-node disease alone above and below the diaphragm. When we say $\mathrm{III}_{\mathrm{E}}$, we mean lymph nodes and localized extralymphatic focus. But when we say $\mathrm{III}_{\mathrm{S+}}$, that can be rather confusing, because it really could be $\mathrm{III}_{\mathrm{S+N-}}$, or it could be $\mathrm{III}_{\mathrm{S+N+}}$. This is an important issue. He therefore urged that we designate specifically whether the lymph nodes in the abdomen are involved as well as the spleen, or the spleen alone.

K. Musshoff explained that the data mentioned in his report at Ann Arbor concerning the prognostic influence of localized extralymphatic disease were based on his statistics in the period from 1948 to 1964. He was now able to report on the therapeutic results recorded in patients examined and treated with modern techniques of megavoltage radiotherapy from 1964 to the present. Of 17 patients classified as Stage IV according to the Rye classification, 9 had a localized extralymphatic organ involved; in the other 8 patients, disseminated extralymphatic organ involvement was diagnosed. In the patients having localized extralymphatic organ involvement the 3-year survival rate was 88%, while it was only 5.6% in patients having disseminated involvement. The corresponding relapse-free survival rates are 41% and 0%. The survival rate and the relapse-free survival rate of the patients with localized organ involvement are similar to the survival rates of the patients with lymph-node disease of corresponding extent, as was the case with a larger number of cases in the period from 1948 to 1964. Thus, these new data indicate that the Ann Arbor staging proposals concerning the classification of localized extralymphatic organ involvement apply also to the conditions of modern radiotherapy.

P. P. Carbone then said that, if there were no other questions or comments about the initial staging of patients using the Ann Arbor classification, the panel could turn to the question of restaging, i.e., staging after recurrence or relapse. He believed that this is an important issue. How are we going to identify whether a stage designation refers to the initial stage or the subsequent stage? **Ultmann** replied that in his study none of the patients that were Stage IV initially were restaged. The initial stage of those later restaged generally was Stage I or II, and very rarely Stage III. **Tubiana** asked whether there would be two stagings for the patient: the initial stage and a subsequent postrelapse stage? **Ultmann** replied that he did not mean that the initial stage could later be changed, for that would create an impossible situation. Once a patient has been staged he has to remain in the same stage until his death. **J. J. Nickson** suggested that a term other than "stage" be used to refer to the subsequent clinical situation, perhaps the term "extent." He agreed that for statistical analysis the initial stage is the one that counts and must remain unchanged. **Ultmann** agreed, but said that, when you have a patient who has had involved field radiotherapy, after staging with or without laparotomy several years ago, and who now reappears with evidence of disease, for example, with a positive lymphogram, we think that clinically at this point they are equal to initially staged cases of Stage III disease, not for statistical purposes but merely for classification and therapeutic decisions.

Tubiana, disputing this view, said that such a case is not equivalent to a Stage III; it is something very different. He stressed the need to recognize the distinction between the different types of relapse after radiotherapy. There are at least 3 different types of relapse: *a*) local or regional lymph-node relapse on the same side of the diaphragm, *b*) lymphatic relapse on the other side of the diaphragm, and *c*) extranodal relapse, either as a localized lesion or general dissemination. If these latter cases were subdivided, one would have 4 types of relapses. **Rosenberg** added that there are other variables in addition to the site or the kind of relapse. Another is the kind of therapy. There is more therapy than just radiotherapy. Chemotherapy may also be given, and then one has a different connotation in a relapse. There is also the problem of the time to relapse. It might make a great deal of difference as far as the prognosis is concerned whether it is a few months or many years. The dose of radiotherapy also comes in, so that one has a very confusing array of variables. He doubted that we are going to be able to resolve it with some simple system and felt that each relapsing patient will be individualized. The closest one might come to it, given those concerns, is that one could add a third stage designation, *RS* for relapsing stage, to the *CS* and *PS* categories now used in the Ann Arbor classification.

SESSION 4

SMALL GROUP WORKSHOPS

Chairmen: **Robert W. Miller, Louis B. Thomas, John E. Ultmann, and B. van der Werf-Messing**

Report on Workshop: Epidemiology, Virology, and Immunology[1]

Robert W. Miller, *Epidemiology Branch, National Cancer Institute,*[2] *Bethesda, Maryland 20014*

H. S. Kaplan outlined the rationale for studying *Epstein-Barr virus (EBV)-induced seroconversion in relation to subsequent Hodgkin's disease.* Among 235 persons 15–34 years old with the neoplasm, his group found that 39 had strong histories and another 9 had suggestive histories of infectious mononucleosis (IM) up to 9 years before (average 4–5 yr). There was no specificity as to the cell type of Hodgkin's disease. Some histories of IM may have been missed because questioning of patients was not uniform in the past as it is now. To determine if a proportion of Hodgkin's disease developing in persons after 15 years of age—say 10–15% of all cases—is related to EBV, Kaplan suggested a prospective study of university students or the military. The sample group would have blood drawn at the beginning of the study, and those who were seronegative then would be re-bled annually for 5 years to see if they became seropositive. The frequency of Hodgkin's disease would be compared among those persons who remained seronegative throughout, those who were seropositive at the beginning, and those who converted during the 5-year interval. It was pointed out that a major problem will be sample size. If 10% of Hodgkin's disease is related to seroconversion during the previous 5 years, millions of persons must be studied. If one is looking for a twofold increase in Hodgkin's disease among persons who seroconvert, the sample must consist of 250,000 who were seronegative at the outset.

It was asked if groups who are genetically at high risk of developing Hodgkin's disease might be used to shrink the sample size (e.g., certain HL-A serotypes or persons with inborn immunologic deficiency diseases, i.e., ataxia-telangiectasia and Wiskott-Aldrich syndrome). The response was that Hodgkin's disease is not frequent enough among patients with genetically transmitted, immunologic deficiency disorders. Among 47 children in this category with lymphoma, only 3 had Hodgkin's disease, according to R. A. Good's statement earlier in this Symposium. It was asked why EBV-induced seroconversion should be studied with regard to Hodgkin's disease and not reticulum cell sarcoma. The response was that 3 bits of evidence point more to Hodgkin's disease than to other lymphomas: 1) Kaplan's observation as described above, 2) the occurrence of Reed-Sternberg-like cells in 5 atypical cases of IM, and 3) the case-contact-case "spread" of Hodgkin's disease which parallels the supposed mode of spread of IM.

Several participants suggested the study of a control group, however imperfect, to be compared with the 235 patients with Hodgkin's disease from whom histories of IM had already been obtained. The information might come from the Stanford University students and faculty who are 15–34 years old. The results would be biased toward diminishing any difference between cases and controls, because presumably the students and faculty would have had better diagnoses or better memories than the less economically favored case group. If the difference persisted, one could be reassured that the frequency of IM observed among the cases was worthy of further study.

Case-contact-case clusters of Hodgkin's disease were described by **J. N. P. Davies.** His group has now investigated about 16 such clusters, with results similar to but less striking than those in Albany, New York. Davies summarized the transmission patterns in Albany, Buffalo, and Mount Kisco. He attempted to use as controls patients with thermal burns, but found nothing comparable to the case-contact-case configuration of Hodgkin's disease. The experience to date indicates that young persons transmit to young, and young to old, but not old to young. The closer the contact, the shorter the latent period. It was shortest for the few case-case contacts studied.

Would one expect case-contact-case clusters to occur when case-case clusters do not? The virologists present thought so. They believed that genetically or immunologically protected carriers may contact many people, some of whom are genetically and immunologically susceptible, whereas a case's contact with sibs is too limited in number. But, it was asked, does not the long duration of contact with sibs and their greater genetic similarity to cases outweigh the greater number of persons of diverse genetics contacted by carriers? The virologists thought not. They believe that IM is transmitted more often by carriers than directly from case to case.

Davies was asked if the carriers had been studied as to HL-A serotype, EBV, and other virus serum reactivity and if blood had been stored for crucial studies in the future. He said that the carriers were very sensitive, and the probability of emotional trauma in asking these carriers to submit to such tests had contraindicated them.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

It was suggested that studies be made to determine if Hodgkin's disease occurs excessively in marital partners of persons with the neoplasm, in children whose mothers had Hodgkin's disease during pregnancy, or in time-space clusters (through the use of new statistical procedures). Even if the results were negative, they would clarify what does not happen and thus refine understanding as to how the neoplasm is transmitted. In studies of clusters, one must remember to pay particular attention to the distribution of cases among persons <40 years old, as in Davies' study.

In perspective, the hypothesis that Hodgkin's disease is transmitted through an asymptomatic carrier, although provocative and in accord with current viral concepts, has not been statistically tested to determine if these occurrences are due to chance.

S. E. Order considered *the future for studies of tumor antigens.* Fast- and slow-migrating antigens have been identified in Hodgkin's disease infiltrates. With purified antisera plus newer technologic methods, it should be possible to determine: 1) discrete lymphocyte antigens in lymphocyte infiltrates, 2) the presence or absence of thymus-related antigens in lymphocyte infiltrates, and 3) abnormal antigens on lymphocytes and/or reticulum cells.

To do so, study must be made of cell layers using the present method of *a*) bovine serum albumin discontinuous gradients, *b*) immunoelectrophoresis, and *c*) immunofluorescence. Availability of antisera prepared against the purified antigens will allow testing of sera and buffy coats of patients. It would be well to begin now storage of such specimens obtained before, during, and after treatment to be used for competitive radioimmunoassay.

To promote understanding of other possible antigens, a thymus (neonatal) antiserum and lymph-node antisera should be prepared and absorbed with various other tissues (liver, spleen, and lymph nodes) to detect cross-reactivity and specific cellular antigens.

With these tools, prognostic and pathologic information about Hodgkin's disease may be derived which will aid in the management of patients and in the detection of the reactivation of disease.

Report on Workshop: Experimental Cytology and Histopathology [1]

Louis B. Thomas, *M.D., Laboratory of Pathology, National Cancer Institute,*[2]
Bethesda, Maryland 20014

The experimental cytologists and pathologists in this workshop first proposed a number of topics and questions which they wished to consider and discuss. The list started with several basic questions about the nature and origin of the neoplastic cells in Hodgkin's disease. A number of other topics were suggested including: 1) questions about the types of reactive hyperplasia in lymph nodes in patients with Hodgkin's disease; 2) the relationships of Hodgkin's disease to other types of malignant lymphomas; 3) the association of the number of lymphocytes in Hodgkin's disease with length of survival; 4) questions about the meaning of non-necrotizing granulomas which are seen in lymph nodes, spleen, and liver sometimes in juxtaposition to Hodgkin's disease tissue and sometimes in areas of the tissue not involved by Hodgkin's disease; 5) questions about observer disagreement and histologic criteria for the diagnosis and subclassification of Hodgkin's disease; and 6) questions about the types of cells grown in tissue culture and, in particular, whether these can be identified as neoplastic cells. In fact, the list of topics proposed for discussion became so long that it was readily apparent that only a few of them could be discussed in the time allotted for the workshop.

It was agreed, therefore, to spend a major portion of the time discussing the first-listed topic: the nature and origin of the neoplastic cells in Hodgkin's disease. **C. Berard** started the discussion by reminding the group that there is considerable evidence that Hodgkin's disease is in some way associated with cellular immunity. Therefore, the proposition that Hodgkin's disease is a neoplastic alteration of the thymus-dependent lymphoid system should be examined. The observation that Hodgkin's disease arises in the perifollicular zone of lymph nodes supports this concept. **M. H. Bennett** then showed several sections of spleens in which Hodgkin's disease cells were located in the peripheral portion of the malpighian follicles. This area too is thought to represent a thymus-dependent zone of lymphoid tissue. Thus there is topographic and anatomic evidence that Hodgkin's disease is first apparent or first recognizable in thymus-dependent areas of lymphoid tissue.

These observations do not answer the question about the cell of origin of Hodgkin's disease because there are many types of reticulum cells, stromal cells, macrophages, and histiocytes in these same areas of the lymph node and spleen. It does seem useful, however, to ask the direct question of whether Hodgkin's disease arises from lymphocytes in these areas. **R. F. Dorfman** then showed several slides of Hodgkin's disease stained with methyl green-pyronine. The cytoplasm of the Hodgkin's mononuclear cells and Reed-Sternberg cells was strongly pyroninophilic. From this and other histochemical evidence, he believes that Hodgkin's disease cells and Reed-Sternberg cells are more like altered lymphocytes than histiocytes. **R. Lukes** then showed some pictures of phytohemagglutinin-transformed lymphocytes and suggested that these transformed cells had cytologic features similar to those of the mononuclear cells of Hodgkin's disease. He concluded that this was at least indirect evidence that Hodgkin's disease cells might be transformed, neoplastic variants of lymphocytes. Against this concept is the fact that pathologists and cytologists have not shown a good or convincing series of cells which cytologically can be interpreted as representing all the intermediary forms between normal lymphocytes and Hodgkin's disease cells. **H. Rappaport, L. M. Schiffer,** and others participating in the discussion believed this weakens the concept that Hodgkin's disease cells are derived from lymphocytes.

J. H. Frenster pointed out that with a tumor-associated, or better yet a tumor-specific antigen, one should be able to determine which are the neoplastic cells and their precursors by a variety of means. He and others discussed how to utilize the methods of cell separation and immunofluorescence once a tumor-specific antigen is purified. **C. P. Bieber** pointed out that these methods are not presently satisfactory because a tumor-specific antigen has not been demonstrated for Hodgkin's disease. The questions of DNA synthesis and cellular division of Reed-Sternberg cells were briefly discussed. From the work presented by Doctors Peckham, Kadin, and Schiffer, the former was felt to be established, but cell division of Reed-Sternberg cells has not been established.

In conclusion, our group did not settle the question about the origin of Hodgkin's disease cells or the related question of how many cells in Hodgkin's disease tissue are neoplastic. Answers to these questions will only be forthcoming after new techniques for identifying cells are developed.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

W. Oehlert started the discussion of the next topic by showing a variety of nonspecific infiltrates in the portal tracts of the liver and raising questions about their significance. **A. Engeset** wondered whether these nonspecific infiltrates were related to lymphangiography or whether they are seen more frequently in the livers of patients who have had lymphangiograms. There was a general consensus that they were not associated with lymphangiography and that the contrast material used is not carried into the liver. It was agreed that interpretation of liver biopsies is difficult because of the sampling problem and because Reed-Sternberg cells are infrequently found. The group did not discuss this matter further but was referred to the report on this problem which was published in the Ann Arbor conference proceedings.

The question of the relevance of the tissue culture work reported at the meeting was brought up and discussed by **Dorfman** and by **A. D. Mitchell.** One difficulty with interpretating the results of these studies is the identification of the cultured cells. Among suggestions offered was that more effort should be made to transplant cultured cells, particularly cell lines that grow actively and appear to be neoplastic, into susceptible animals.

Bennett then presented some data on karyotyping Hodgkin's disease cells obtained from lymph nodes and spleen. In one instance he found an identical marker chromosome in tissue from 2 lymph nodes from the same patient and interpreted this as evidence for clonal origin and dissemination of Hodgkin's disease cells. Problems in the interpretation of data from chromosomal studies were then briefly discussed by **C. P. Miles.** It was agreed that these types of study should be continued but that the results will be more valuable when better methods of cell separation and cell identification are developed.

H. Rappaport then presented his data showing that the number of lymphocytes in histologic sections of nodular-sclerosing and mixed-cellularity Hodgkin's disease correlated directly with survival. Other cells did not correlate to a significant degree. In the discussion, it was pointed out that the grouping of cases according to number of lymphocytes had not been adjusted for stage. It is probable that a low percentage of lymphocytes in the lesions will be found to correlate with more advanced stages of disease and with a decreased number of circulating lymphocytes.

Report on Workshop: Clinical Evaluation, Staging, and Prognosis [1,2]

John E. Ultmann, *Department of Medicine, Section of Hematology, University of Chicago, Pritzker School of Medicine, Chicago, Illinois 60637*

The first part of the meeting was devoted to a discussion of new techniques in evaluating extent of disease and assessing the host's immunologic status.

M. Tubiana inquired regarding present research in *assessing immunologic responsiveness.* He commented regarding the discrepancy between various reports depending on patient material studied and techniques employed. It was recommended that a careful definition of immunologic responsiveness of a control population be undertaken and that techniques thus developed be applied to untreated, carefully staged patients with Hodgkin's disease.

S. B. Strum raised a question regarding use of lung-mediastinal *tomography.* Although ideally this technique might be employed routinely, its use of necessity must often be restricted. It is particularly important to perform when routine posterior-anterior and lateral roentgenologic examinations show abnormalities. With bilateral neck involvement, there is a great likelihood that the mediastinum is involved. Whole-lung tomography may also be helpful in patients with clinical symptoms and elevated erythrocyte sedimentation rate when other tests are negative.

The usefulness of *bone marrow biopsy* was reviewed by **S. A. Rosenberg,** who summarized his previously reported data.[3]

No new information regarding predictive parameters for *liver involvement* (other than splenic involvement at laparotomy) was developed.

P. B. Hoffer reviewed his experience with *^{99}Tc scans of bone marrow* performed in conjunction with liver-spleen scans. The cost-time factor appears small. Correlation in individual cases with the ^{18}F scan was stressed. There are no studies reported correlating radiologic and isotope studies of bone and bone marrow with biopsy results, and such an investigation should be encouraged.

R. A. Castellino reviewed the Stanford University experience with 33 patients who had *splenic arteriography* in an attempt to predict splenic involvement. This was found not to be a useful procedure.

It is estimated that over 2,000 *laparotomies and splenectomies* for staging Hodgkin's disease have been performed to date. Current reports stress the diagnostic value of this procedure. However, there is little information available regarding the immediate and long-term risks. It was proposed that a canvass of university, as well as community, hospitals be undertaken to compile the incidence of complications—operative, postoperative, and long-range. The risk-reward ratio, particularly in hospitals having little experience with this procedure, should be determined.

The second part of the meeting consisted of a discussion of the usefulness of staging patients in relapse. It was emphasized how important precise restaging is in determining treatment strategy. It is recommended that the current nomenclature developed for primary staging (Ann Arbor Classification) *not* be applied to patients in relapse.

For patients who have had *only radiotherapy* in the past and who are found to be in relapse, the following designations are suggested:

> *Local recurrence* (LR or TR) [4]—This is a recurrence in an area previously treated.[5] Tumor dose of RT previously given should be specified.
>
> *Regional recurrence* (MR, Adj R) [4]—This is a recurrence outside the previously treated area [5]; however, it is confined to the same side of the diaphragm where disease was found initially.
>
> *Transdiaphragmatic recurrence* (TDR) [4]—This signifies appearance of disease in lymph nodes (or spleen) but on the other side of the diaphragm than originally noted.
>
> *Extra-lymphatic recurrence* (EL) [4]—This is the appearance of disease in extra-nodal or extra-splenic sites and may be designated as L+, H+, M+, D+, etc.

For patients who have had chemotherapy or chemotherapy and radiotherapy, the relapse designations should be kept very simple:

> *Recurrence*—This designates reappearance of disease in one or multiple sites as observed during prior staging.
>
> *Local problems*—These should be designated specifically when they appear in a patient following administration of chemotherapy.

The panel stressed the importance of staging in relapse to determine accurately treatment strategy. Additional biopsies, including occasionally laparotomy, are considered useful.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Chairman: Dr. Ultmann. Committee Members: Paul P. Carbone, Ronald A. Castellino, Paul B. Hoffer, A. M. Jelliffe, Henry S. Kaplan, Saul A. Rosenberg, and M. Tubiana.

[3] Rosenberg SA: Hodgkin's disease of the bone marrow. Cancer Res 31:1733–1736, 1971.

[4] More than one type of recurrence may be present in a single patient. Each type should be coded.

[5] At times, it is difficult to differentiate a local recurrence from a marginal recurrence.

Report on Workshop: Chemotherapy[1]

Paul P. Carbone, *Medical Oncology, National Cancer Institute,*[2] *Bethesda, Maryland 20014*

The chemotherapy workshop was attended by a small group representing Stanford University, Stanford, California; Middlesex Hospital, London, England; Milan Cancer Institute, Milan, Italy; Royal Marsden Hospital, Surrey, England; and the National Cancer Institute (NCI), Bethesda, Maryland. The session was chaired by V. T. DeVita. The discussion considered chemotherapy, current results and new agents, criteria for response and remission, and design of clinical trials.

G. Bonadonna reported new drug studies from the Milan Cancer Institute with adriamycin and bleomycin in patients with malignant lymphoma. He reported good clinical responses in 75% of 24 patients with Hodgkin's disease and 70% of 30 patients with non-Hodgkin's lymphoma. He recommended 20–25 mg/m^2 of adriamycin given daily for 3 days and repeated every 2–3 weeks as an effective and tolerable dose schedule. He was not able to recommend a dosage schedule for bleomycin.

Data were presented regarding cyclohexyl-chloroethylnitrosourea (CCNU) from the Acute Leukemia Group B that showed this agent to be highly effective in patients with Hodgkin's disease, more so than 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). However, the data may be biased by sample differences between the 2 groups in favor of CCNU.

Although these agents appear to be promising, showing evidence of activity when other modalities fail to work, the consensus was that except for bleomycin, marrow toxicity was often limiting. Many patients who are to receive these new drugs have had a great deal of prior treatment and have compromised marrows. Thus, one of the recommendations of the Chemotherapy Workshop will be the development of drugs that have primarily little marrow toxicity.

The experience with the MOPP combination (mechlorethamine, vincristine, procarbazine, and prednisone) has been amply confirmed as to the high response rate. Toxicities, primarily marrow and neural, have created some degree of doctor and/or patient reluctance. Moreover, a newly recognized complication seems to be azospermia in male patients undergoing MOPP treatment. Suggested remedies include sperm banking and possibly inhibition of spermatogenesis by concomitant administration of androgens to prevent toxicity to dividing sperm cells. A second complication seen at the NCI is related to an apparent increase in second malignancies in patients undergoing combination chemotherapy following relapse after total-nodal radiotherapy. These tumors include a fibrosarcoma, epidermoid carcinoma, and a mucinous adenocarcinoma, all occurring in young people. The overall incidence of second malignancies in radiotherapy-treated or combination chemotherapy-treated patients was $<3\%$. The high incidence in the combined treatment program is alarming. The patients currently under treatment at Stanford University have not had a high incidence of second tumors, but most of these patients have had <2 years of observation.

While the complete remission rate has been amply confirmed by several clinical trials with combination chemotherapy, the remission duration has varied considerably from <6 months at Stanford University to >2 to 3 years from reports of the Southwest Group and the NCI. Some of the reasons for these study differences are undoubtedly related to lack of uniformity in the definition of complete response, particularly relating to remission evaluation. Another explanation obviously may be related to the differences in the mix of patient populations admitted to the study.

Disease-free survival is the ultimate goal of all treatment. While the only long-term results available are still from the NCI where 40% have had no evidence of disease at 4 years, obviously most patients still have relapses. Thus, we must continue to search for other combinations that can be included sequentially, hoping to evaluate a resistant population or employ other modalities such as immunotherapy.

A major problem relates to the optimal duration of treatment. Most centers continue to employ the arbitrary 6 months' therapy regimen. This, in fact, has been modified at the NCI to continue treatment for at least 2 additional cycles beyond complete remission or a minimum of 6 cycles. Some patients have required 8 or 9 cycles. A related question pertains to the value of continued treatment or maintenance. The study at the NCI fails to show convincingly the value of additional treatment with MOPP or BCNU. On the other hand, the Southwest Cooperative Chemotherapy Group and the Acute Leukemia Group B both find advantages of additional treatment. We must come up with a better method of determining complete remission. This possibly can be done by using a biochemical marker such as alpha fetoprotein or human choriogonadotropin.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Thus, the recommendations of the Chemotherapy Workshop are as follows:

I) A standard definition of complete remission must be adopted. We recommend that a complete response be reported only when the patient has a normal physical examination and no evidence of disease-related symptoms, all previously abnormal tests have become normal, and repeat examination by biopsy if possible of major organ sites, such as the bone marrow and spleen, has returned to normal.

2) In the assignment of patients to treatment programs, proper controls and assignment of patients are necessary. For stratification of patients between treatment programs, we must take into account prior therapy, stage, histology, age, sex, and, possibly, site of organ involvement.

3) New trials should consider a comparison of new therapy to the best available program whenever possible with crossover of nonresponders to the other treatment and responders to see if the new treatment will affect a resistant subpopulation, thereby providing for additional cell kill. As a measure of cell kill, a no-maintenance treatment program will provide some estimate by the relative duration of disease-free survival.

4) For chemotherapy-alone programs, we should treat Stage IV-A and IV-B primarily and possibly III-B. Patients with Stages III-A, III-B, and II-B would probably benefit from combined X-ray and drug approaches. The sequence of the treatment programs may prove important. So far, the program of using radiotherapy followed by chemotherapy appears to be better tolerated with MOPP; however, other combinations may be useful prior to radiotherapy.

5) Chemotherapy and radiotherapy trials carry some long-term hazards: leukemogenesis, sterility, fibrosis, etc. A central registry and careful compilation of the true risk must be set up to detect as soon as possible undue hazard of serious toxicity.

SESSION 5

TREATMENT

Part 1. Current Status of Clinical Trials

Chairman: M. Tubiana

INTRODUCTION

With the discussion of current clinical trials, I believe that we are reaching the root of the matter. I think it is appropriate to remind the audience that the first randomized clinical trials on Hodgkin's disease began here at Stanford University about 10 years ago. Most of the clinical trials which have been initiated elsewhere since that time were stimulated by the achievements of Henry S. Kaplan and his group, and this is something we are all glad to acknowledge. But the importance of this accomplishment is not limited to Hodgkin's disease. What several major centers in the United States and Europe have done to change the prognosis in Hodgkin's disease, which 30 years ago seemed a hopeless disease, can be used as a model for other cancers. Let us hope that the same attack will have similar success in other cancers and let us hope also that this discussion will help further clarify for us the treatment of Hodgkin's disease.

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Current Status of Clinical Trials: Stanford Experience, 1962–72 [1,2]

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SUMMARY—The first 2 randomized clinical trials involving patients with Hodgkin's disease were initiated at Stanford University Medical Center in May 1962; several other trials were established subsequently. This paper presents an actuarial analysis of survival and of freedom from relapse to date in these trials. Total-nodal radiotherapy yielded a significantly lower relapse rate than local, involved-field radiotherapy in patients with disease of Stage I-A or II-A extent (Ann Arbor classification), but survival does not differ appreciably in the 2 groups. In contrast, when constitutional symptoms were present in these stages (I-B, II-B), total-nodal radiotherapy was significantly better than involved-field radiotherapy with respect to both survival and fredom from relapse. A more recent trial has tested the value of supplementing total-nodal radiotherapy with combination chemotherapy, relative to total-nodal radiotherapy alone, in Stages I-B, II-B, III-A, and III-B. After 3 years, the combined treatment group exhibits a survival rate of 100%, with no relapses, as compared with a 92.8% survival rate and 29% relapse rate (neither value being statistically significant) among the group treated with radiotherapy alone. Finally, patients with Stage III_S-A and III_S-B disease, in whom splenic involvement has been documented at laparotomy, have been treated with total-nodal radiotherapy supplemented with either combination chemotherapy or hepatic irradiation (external plus intravenous ^{198}Au-gold colloid). The observed relapse rates are not significantly different, and both groups have had remarkably high survival (about 85% at 4 years).—Natl Cancer Inst Monogr 36: 363–371, 1973.

DURING THE LAST 2 decades, the randomized clinical trial has emerged as a powerful, rigorous, and objective method for the assessment of the relative merits of different approaches to the treatment of various types of cancer. The first randomized clinical trials in Hodgkin's disease, to the best of our knowledge, were the 2 trials established at Stanford University Medical Center in May 1962, stemming from the highly encouraging results with intensive megavoltage radiotherapy obtained in an earlier nonrandomized study (*1*). In the course of time, these 2 randomized trials stimulated similar clinical investigations at a number of other centers in the United States and abroad. The collective experience emerging from these early trials resulted in turn in the design of a series of new trials initiated at Stanford in 1968 and 1970. This paper presents the data to date with respect to survival and freedom from relapse in all the Stanford clinical trials, with the exception of those in which the numbers of accessions and the interval available for follow-up seemed insufficient to provide meaningful information.

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MATERIALS AND METHODS

The general pattern of the randomized trials conducted at Stanford was described previously (*2–4*). The first 2 trials had the following objectives: *a*) to determine whether intensive irradiation of involved lymph-node chains and adjacent, apparently uninvolved chains of nodes (extended-field or EF radiotherapy) in patients with regionally localized Hodgkin's disease increases the duration of remission and of survival significantly relative to that afforded by similar doses of radiation confined to the involved nodes alone (involved-field or IF radiotherapy); *b*) to determine whether the use of potentially curative doses of radiation to all major lymphoid structures (total-nodal or TN radiotherapy) given to patients with generalized (Stage III) Hodgkin's disease is more effective than the use of localized, palliative-dose radiotherapy as measured by length of remission and/ survival; *c*) to accumulate additional information on the natural history of Hodgkin's disease in patients whose clinical stage was routinely determined with the aid of lymphangiography and other special diagnostic procedures; and *d*) to evaluate the tolerance of normal tissues in patients treated with EF, high-dose radiation.

The definition of clinical stages has undergone an appreciable evolution during this past decade, beginning with our 4-stage modification (2) of the well-known clinical staging classification of Peters and Middlemiss (*5*), which, with some further modifications, later became the Rye 4-stage classification (*6*). More recently, additional modifications in the clinical staging of Hodgkin's disease, which are also applicable to the other malignant lymphomas, have been proposed at Stanford (*7*) and at Ann Arbor (*8*).

In this paper, all stage allocations conform to the Ann Arbor clinical staging classification (*8*). However, in the initial clinical trials, the Stanford (1964) and subsequent Rye Stage IV definition (*6*) was used as the basis for exclusion of patients with extralymphatic disease (so-called "E" cases in the Ann Arbor scheme) from either of the 2 initial clinical trials. Other criteria for exclusion of patients from these trials included: lack of confirmation of the initial biopsy diagnosis by our own surgical pathologists at Stanford; previous definitive treatment, other than biopsy; the presence of another primary malignant neoplasm or other life-threatening disease; lack of informed consent by the patient, guardian, or the referring physician; and domicile more than 400 miles from the Stanford University Medical Center.

All other patients with biopsy-proved, previously untreated Hodgkin's disease living within the defined geographic area were accepted for one or the other of these 2 randomized clinical trials. They were routinely subjected to thorough diagnostic evaluation as described previously (*2, 4*), including lymphangiography, a battery of liver function tests, and needle and/ or open bone-marrow biopsy. The clinical (and later pathologic) stage was determined from the information provided by these studies. Patients in the relevant stages of a given protocol were then allocated to treatment options A or B, according to the instructions contained in a randomly assorted series of sealed envelopes. The same general protocol design has been used in all the subsequently adopted clinical trials, modified only by the routine utilization of staging laparotomy with splenectomy (*9, 10*) since September 1, 1968. The scope of the clinical trials was recently broadened to include Ann Arbor Stage IV cases, and the treatment options in some of the new protocols have reflected the highly encouraging experience of the Bethesda group (National Institutes of Health) with quadruple drug combination chemotherapy (*11*). The clinical trials which have been in operation at Stanford University Medical Center, their dates of initiation, the clinical stages represented, and the treatment options are listed in table 1. The techniques of radiotherapy were described previously (*4, 7*); except for the palliative dose group of Stage III cases in protocol L-2, who received 1500 rads in 2 weeks to involved lymph-node chains only, the usual dose range in all other cases, to both involved and uninvolved regions, was 3500–4400 rads, delivered at a rate of 1000–1100 rads per week.

All patients were carefully followed in a weekly conjoint clinic and seen at gradually decreasing intervals, starting with visits every 1–2 months during the 1st year after completion of treatment and gradually increasing to yearly

TABLE 1.—*Stanford randomized clinical trials in Hodgkin's disease*

Protocol	Year initiated	Stage(s) represented	Treatment option*	
			A	B
L-1	1962	I and II (A+B)	IF radiotherapy (4400 rads/4 wk)	Limited EF radiotherapy (4400 rads/4 wk)
L-2	1962	III (A+B)	Low-dose (1500 rads/2 wk) IF radiotherapy	High-dose (>3500 rads/4 wk) TN radiotherapy
H-1	1967	I-A and II-A	IF radiotherapy (4400 rads/4 wk)	TN radiotherapy (4400 rads/4 wk)
H-2	1968	I-B and II-B	TN radiotherapy only (4400 rads/4 wk to involved sites, 3500–4400 to uninvolved)	TN radiotherapy, then MOPP (6 cycles)
H-3	1968	III (A+B)	TN radiotherapy only (4400 rads/4 wk to involved sites, 3500–4400 to uninvolved)	TN radiotherapy, then MOPP (6 cycles)
H-4	1968	I_E-B, II_E-B, III_E (A+B)	TN radiotherapy only (4400 rads/4 wk to involved sites, 3500–4400 to uninvolved)	TN radiotherapy, then MOPP (6 cycles)
H-5	1968	II_S (A+B) III_S (A+B)	TN radiotherapy and liver irradiation (combined external and ^{198}Au-gold colloid)	TN radiotherapy, then MOPP (6 cycles)
H-6	1968	IV_H (A+B)	TN radiotherapy + liver irradiation	MOPP alone (6 cycles)
K-7	1968	IV_M, IV_L, etc.	"Split course" MOPP (2 cycles before, 4 cycles after TN radiotherapy)	MOPP alone (6 cycles)
R-1	1970	I-A, I_E-A, II-A, II_E-A ("favorable" sites)	Mantle or inverted-Y field radiotherapy only (4400 rads/4 wk)	IF radiotherapy (4400 rads/4 wk) + MOPP (6 cycles)
K-1	1970	I-A, I_E-A, II-A, II_E-A ("unfavorable" sites)	Subtotal radiotherapy (pelvis omitted for supradiaphragmatic involved sites; 4400 rads/4 wk)	Subtotal radiotherapy + MOPP (6 cycles)

*IF = involved field; EF = extended field; TN = total nodal; MOPP = a combination of mechlorethamine, vincristine, procarbazine, and prednisone.

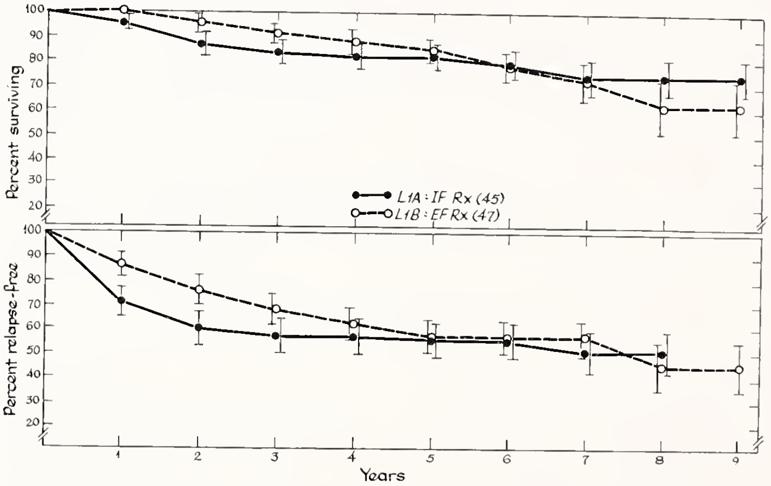
visits after the 5th year. At each visit, routine chest and abdominal radiographic examinations, complete blood counts, erythrocyte sedimentation rates, and serum alkaline phosphatase determinations were obtained, in addition to an interval history and physical examination. An attempt was made, whenever possible, to document clinical evidence of relapse by biopsy and histopathologic confirmation before initiation of additional treatment. Data on survival and freedom from relapse were analyzed actuarially by the method of Berkson and Gage (*12*), and tests for the significance of differences between the results of certain trials were made by the method of Gehan (*13*), with the aid of an IBM 360, model 50 computer. Only those tests of significance yielding *P* values ≦5% level are indicated in the text-figures.

RESULTS

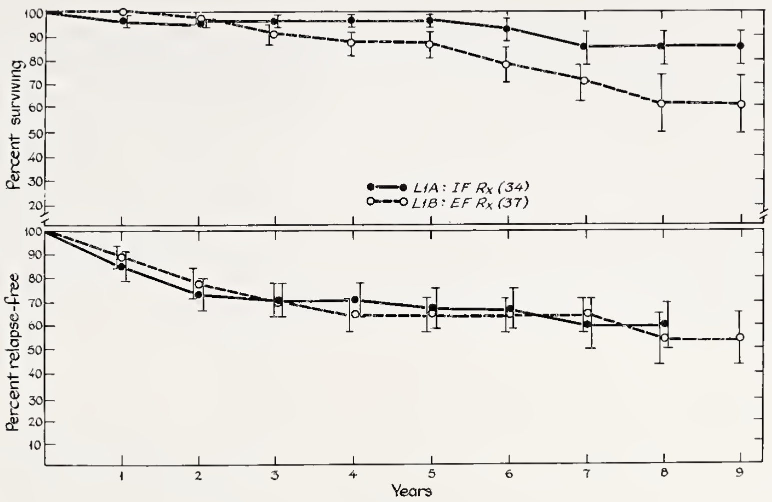
The results of all Stanford clinical trials for which adequate data are available are presented in text-figures 1 through 7. All curves for survival and relapse were terminated when <5 patients remained at risk in any given treatment group.

Text-figure 1 shows that there was no significant difference in survival or freedom from relapse in patients with regionally localized disease (all Stage I and II, A and B cases) treated by the IF or EF techniques of radiotherapy, respectively. Indeed, when one considers only the asymptomatic cases (Stage I-A and II-A) in this protocol, survival was paradoxically somewhat better, though not significantly so, after IF than EF treatment (text-fig. 2). However, EF treatment was significantly better than IF treatment, for both survival and freedom from relapse, in the L-1 protocol cases with constitutional symptoms (Stages I-B and II-B; text-fig. 3).

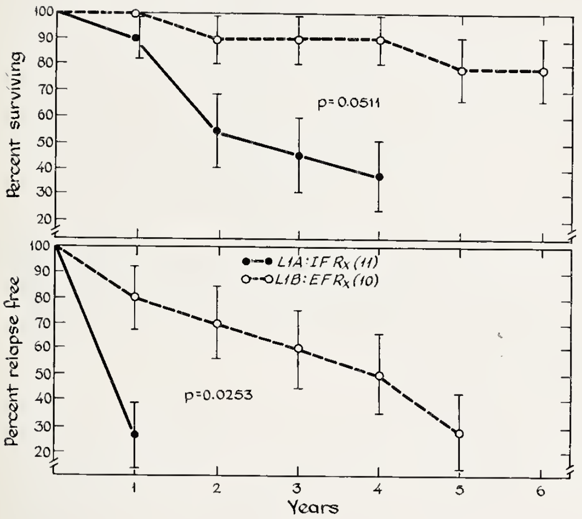
There was an unexpectedly high incidence of relapse in the lumbar para-aortic lymph nodes (*14*) and sometimes also in the abdominal viscera, after either IF or EF treatment in patients whose disease had initially been confined to the cervical, supraclavicular, axillary, and other supramediastinal lymph-node regions. When EF treatment was initially planned, it was thought that the mediastinal nodes were the next chain



Text-figure 1.—Actuarial analysis of survival (*upper panel*) and freedom from relapse (*lower panel*) in patients with Stage I and II (A + B) Hodgkin's allocated at random within L-1 protocol to IF versus EF radiotherapy. *Numbers in parentheses* indicate numbers of patients at risk. Standard error of each point is indicated by adjacent *vertical bar*.



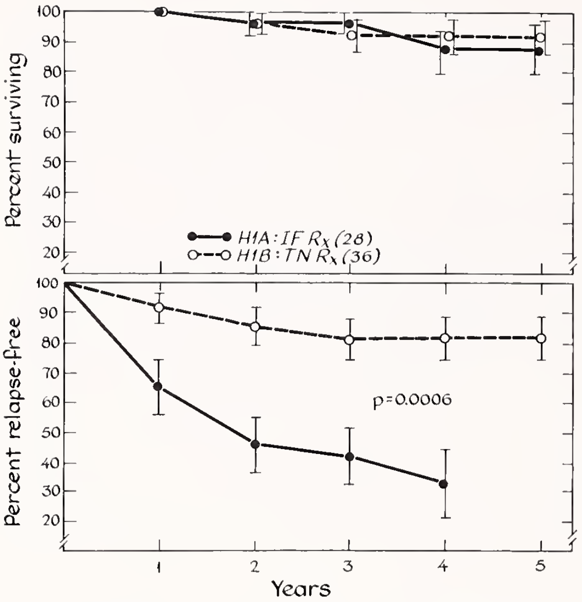
TEXT-FIGURE 2.—L-1 protocol data for asymptomatic cases (Stages I-A and II-A) only. Symbols and other details as per text-figure 1.



TEXT-FIGURE 3.—L-1 protocol data for cases with constitutional symptoms (Stages I-B and II-B) only. Symbols and other details as per text-figure 1. Statistical significance of observed differences in survival and relapse rate, denoted by indicated *P*-values, was computed by the method of Gehan (*13*).

at risk in such situations, and thus that prophylactic treatment below the diaphragm would be unnecessary except in cases with mediastinal adenopathy. With the recognition of the predilection of Hodgkin's disease for spread from the cervical-supraclavicular to the upper lumbar para-aortic lymph nodes, bypassing the mediastinum (*15, 16*), the EF technique, at least in its original limited form, was clearly inadequate to provide the kind of protection against microscopic spread of Hodgkin's disease for which it had been intended. However, under certain circumstances, notably in the presence of mediastinal adenopathy, the EF technique did include treatment of the retroperitoneal lymph nodes down to the level of the iliac bifurcation in females and including the pelvic nodes in males. Because many patients with constitutional symptoms assigned to EF treatment had mediastinal involvement, their treatment was essentially indistinguishable from the TN technique. It is thus of interest that there was a significant difference not only in relapse rate but also in long-term survival in the patients with constitutional symptoms (Stage I-B and II-B) who were treated by the IF versus the EF (+ TN) techniques in the L-1 protocol (text-fig. 3).

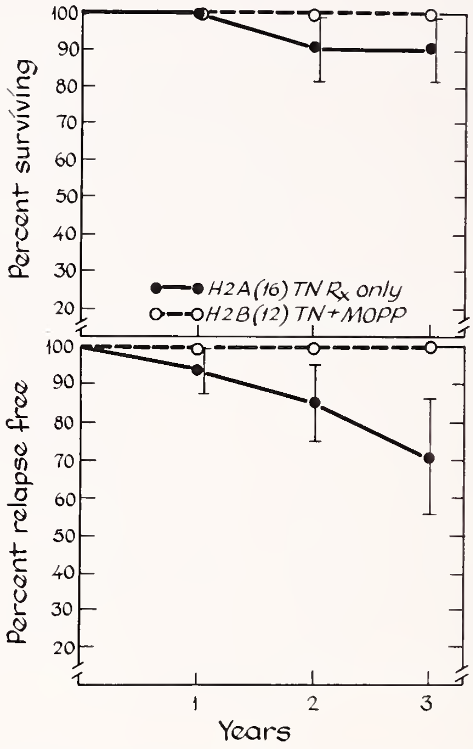
These experiences with the L-1 protocol led us to initiate a new clinical trial, H-1, in July 1967, in which the treatment options in Stage I-A and II cases were more sharply polarized as IF versus TN radiotherapy. This clinical trial was later modified by the routine introduction of staging laparotomy with splenectomy after



Text-figure 4.—H-1 protocol data for IF versus TN radiotherapy in Stage I-A and II-A Hodgkin's disease. Although there is no difference in survival at 5 years, the difference in relapse rates is highly significant ($P = 0.0006$).

September 1, 1968. Analysis of the data to date in the H-1 protocol indicates that, although there is as yet no significant difference in survival between the 2 treatment techniques, a highly significant difference in relapse rate, previously noted in our preliminary data (*7*), has been sustained to date (text-fig. 4).

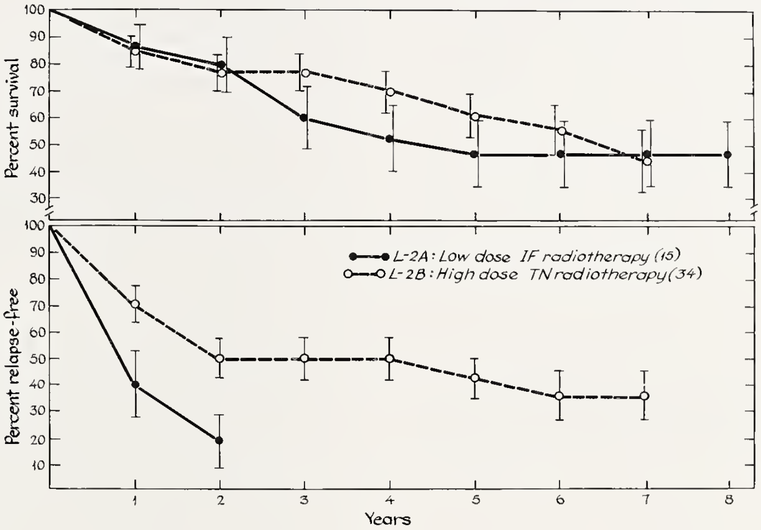
Because of the relatively poor prognosis after IF radiotherapy of the patients with regionally localized disease associated with constitutional symptoms (Stages I-B and II-B), treatment with the TN technique alone was made the new "standard" treatment (option A) for such cases in the new H-2 protocol adopted in 1968. In option B, TN radiotherapy was supplemented, after a rest period of 6–8 weeks, with MOPP quadruple drug chemotherapy. Interestingly, survival and freedom from relapse have both remained at 100% through a full 3 years for those patients allocated to treatment option H-2B, who received both TN radiation and 6 subsequent cycles of MOPP combination chemotherapy (text-fig. 5). In contrast, patients treated with TN irradiation alone in these same clinical stages have suffered a 29% relapse rate and a slight decrease in survival to 92.8% by the end of the 3d year. However, because of the few cases in both arms of the study, neither difference is statistically significant.



Text-figure 5.—H-2 protocol data for patients with Stage I-B and II-B Hodgkin's disease receiving TN radiotherapy alone or supplemented with 6 cycles of MOPP combination chemotherapy. The differences are not statistically significant.

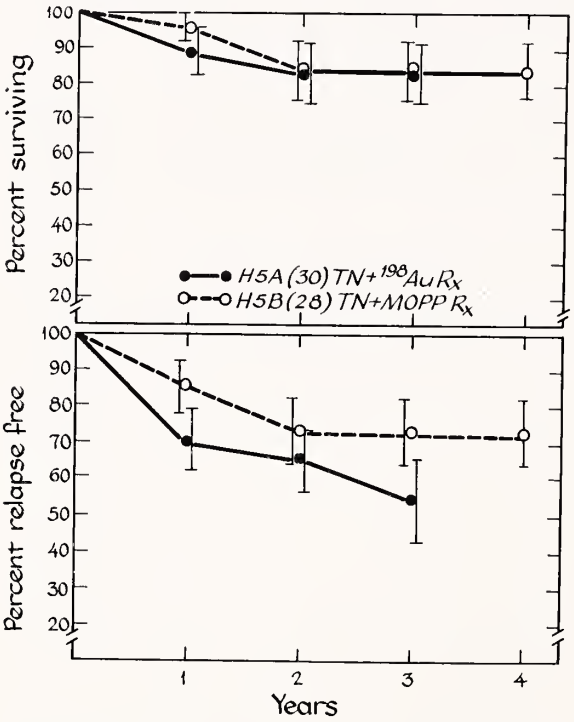
The L-2 protocol was the first one, to our knowledge, in which a deliberate attempt was made to use treatment with curative intent in Stage III (A and B) Hodgkin's disease. At the time of its initiation, TN radiotherapy was an unknown technique, the potential hazards of which were regarded with considerable trepidation. As indicated elsewhere (*4, 17*), patient tolerance for TN radiotherapy proved to be surprisingly good. Because of this tolerance and since the technique seemed to be yielding long-term, relapse-free survival in an appreciable proportion of Stage III-A and III-B patients early in the course of this protocol, the alternative randomization to palliative treatment was terminated in March 1965, 3 years after initiation of the protocol, and all patients subsequently admitted to it were allocated to the L-2B TN treatment option. This explains the differential numbers of patients allocated to the 2 treatment options.

However, because patients initially allocated to treatment option L-2A, which entailed what was regarded as merely palliative local-field radiotherapy, had received treatment clearly inadequate by the standards of our subsequent experience, every effort was made to salvage these patients by subsequent TN radiotherapy and/or MOPP chemotherapy after clinical evidence of relapse occurred. That a substantial degree of salvage was obtained is evident from the survival curves for the L-2A versus L-2B groups, which diverged moderately from the 3d through the 6th years but did not differ significantly thereafter (text-fig. 6). There was, however, a great difference in relapse rate between the L-2A and L-2B groups.



TEXT-FIGURE 6.—L-2 protocol data for patients with Stage III (A and B) Hodgkin's disease. Those in group L-2B were treated with high-dose (3500–4400 rads/4 wk) TN radiotherapy initially, whereas those in group L-2A received low-dose (1500 rads/2 wk) IF radiotherapy initially, followed after their relapse, whenever possible, by the high-dose TN radiotherapy. The observed differences are not statistically significant.

The surprisingly favorable results obtained from radical TN treatment of patients with Stage III-A and III-B disease, considered prognostically hopeless before 1962, led in turn to the new H-3 and H-5 protocols. In protocol H-3, concerned with Stage III cases in which the spleen was not involved, TN radiotherapy alone became treatment option A and was supplemented by 6 cycles of MOPP chemotherapy in option B. In protocol H-5, in which involvement of the spleen had been documented at staging laparotomy, TN radiotherapy was supplemented in option A by whole-liver irradiation with combined external-beam treatment and fractionated doses of intravenous colloidal radioactive gold for presumptive microscopic liver involvement. In treatment option H-5B, as in protocol H-3, 6 cycles of MOPP quadruple combination chemotherapy were initiated after a rest period of 6–8 weeks after completion of TN radiotherapy. As detailed elsewhere (*18*), this formidable combination of radical radiotherapy and radical combination chemotherapy also proved to be surprisingly well tolerated by at least 90% of patients allocated to such treatment, and the initial results were highly favorable. However, there have recently been 4 relapses and 2 deaths in the H-5B protocol group; thus, survival and relapse rates in this group are no longer significantly better than those in the H-5A group (text-fig. 7). Additional periods of observation and the accession of additional patients in both treatment options will be required before the relative merits of these 2 approaches to treatment in Stage III_S-A and III_S-B Hodgkin's disease can be more objectively assessed. The numbers of cases in both arms of the H-3 Hodgkin's disease protocol are still too few to warrant reporting at this time.



TEXT-FIGURE 7.—H-5 protocol data for patients with Stage III_S (A and B) Hodgkin's disease with laparotomy-proved splenic involvement. Those in the H-5A group were randomized to TN radiotherapy plus irradiation of the liver with a combination of external-beam and internally deposited isotope (^{198}Au, given intravenously); those in the H-5B group received TN radiotherapy followed by 6 cycles of MOPP combination chemotherapy. The differences in relapse rates are no longer significant, though they were at an earlier stage in the study (*18*). *Note* remarkably high survival to date (about 85% at 4 yr) with both types of treatment in this series of patients with relatively far-advanced disease.

Finally, the newer clinical trials, H-6 and K-7, for patients with documented Stage IV disease by virtue of liver involvement or of bone marrow and other sites of disseminated involvement, respectively, are still too recent to have accumulated significant numbers of patients for analysis at this time. However, the technique of "split-course" MOPP combination chemotherapy plus TN radiotherapy, usually involving 2 initial cycles of MOPP chemotherapy, then the full course of TN radiotherapy, followed by the resumption of MOPP chemotherapy and subsequent maintenance chemotherapy, has on the whole been quite well tolerated by these Stage IV cases, and preliminary results appear highly favorable.

DISCUSSION

These clinical trials and those conducted in a number of other centers in the United States and abroad have generated a great deal of new information about Hodgkin's disease. There is now little doubt that modern techniques of intensive megavoltage radiotherapy and combination chemotherapy have sharply improved the prognosis of patients with Hodgkin's disease, and mounting evidence indicates that not only long-term survival but also actual permanent cure is being obtained in a high proportion of patients thus treated (*17*).

These trials have also generated a great deal of new information about the natural history and patterns of spread of the disease. In particular, during these trials, it was appreciated for the first time that there is a very high predilection for spread from the cervical-supraclavicular lymph nodes to the upper lumbar para-aortic lymph nodes and/or the spleen (*14*). Moreover, growing experience with staging laparotomy and splenectomy (*19–21*) has confirmed the early Stanford experience (*9, 10*), indicating the hitherto unsuspected frequency with which silent involvement of the spleen may be present in Hodgkin's disease.

The trials have also documented the surprisingly good hematologic and systemic tolerance of patients with Hodgkin's disease for such formidable treatments as TN radiotherapy and MOPP or other combinations of multidrug chemotherapy. Accordingly, even though significant differences in survival and relapse rates were not uniformly observed in all trials in which radical treatment was compared with more limited treatment, there has been a growing tendency to move toward radical treatment in a higher proportion of patients with Hodgkin's disease of all stages. Clearly, what is now needed are clinical or laboratory criteria whereby patients with disease of a given anatomical extent, with or without constitutional symptoms, can be further assessed to ascertain whether they do, in fact, require such aggressive TN or MOPP treatment, or would do equally well to have more limited IF radiotherapy initially, holding radical treatment in reserve for those patients who have relapses. If it were possible to discover in advance which patients would do well with limited treatment, these patients would of course benefit thereby, since they would be spared the rigors of the much more aggressive treatment techniques. There is thus a need, in our ongoing studies during the coming decade, to emphasize the search for valid criteria that can be used to separate those patients requiring only limited treatment from those in whom the more aggressive treatment techniques would provide a significantly better prognosis. It is likely that the histopathologic type of the disease, the presence or absence of constitutional symptoms, and certain laboratory indicators (*22*) may prove valuable in this context.

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Combined Drug Treatment of Hodgkin's Disease: Remission Induction, Remission Duration, and Survival; an Appraisal[1]

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SUMMARY—Several studies employing combinations of effective drugs consistently report an increased rate of complete remissions in patients with advanced Hodgkin's disease. One cooperative study has also confirmed the prolongation of remission observed in the original study from the National Cancer Institute. With a doubling or tripling of the complete remission rate to >50% of patients so treated, and an eightfold to tenfold increase in remission duration after therapy, it is not surprising that survival is prolonged over that obtainable in the past using single agents. Data from the National Cancer Institute show that approximately 70% of the patients who achieved a complete remission with combination chemotherapy between 1964 and 1967 are alive at 5 and 6 years. Of complete responders, 41% have remained continuously free of disease up to 6 years with no further treatment. Differences in complete remission rates and remission duration from study to study can probably be explained by failure to consider variables known to affect a patient's prognosis, e.g., age, sex, site of organ involvement, symptoms, histopathology, and distribution and size of involved lymph nodes. Future studies should stratify the patient populations by these factors. The complete remission rate alone should not be used to judge the effectiveness of a treatment program. Such programs should also be evaluated by their ability to produce a long disease-free interval after all treatment is stopped.—Natl Cancer Inst Monogr 36: 373–379, 1973.

HODGKIN'S DISEASE is often more extensive than first appreciated by physical examination or radiologic and surgical procedures (*1–4*). This observation, coupled with the knowledge that certain histopathologic subgroups have a high risk of dissemination (*5*), emphasizes the need for an effective form of systemic therapy. Single drugs have been ineffective either in inducing a high percentage of complete remissions or, more importantly, in rendering a significant number of patients free of disease for long periods (*6*). Drug combinations have been more effective in accomplishing these goals than single drugs. Long-term follow-up data are now available from the study originally reported from the National Cancer Institute (NCI) (*7–9*). Several cooperative groups have also begun to make the results of their drug combination studies available, although few have been published in detail (*10–13*). A summary of the data of all drug combination studies in progress in the cooperative chemotherapy groups has recently been reported by Carter (*14*).

Interpretation of these varied data continues to be difficult because of the lack of uniform reporting of responses in reference to factors known to influence prognosis, such as age, sex, site of involvement, histopathology, stage, and prior treatment. When randomized clinical trials have been performed, these factors have usually not been considered in the original randomization scheme. Other problems in interpretation of data are introduced by differences in the approach to staging, duration of therapy, interpretation of and accuracy of the term "'complete remission," and planned reduction in dosing in response to toxicity. Despite these impressive numbers of variables, the results have been surprisingly uniform. The data available from several drug combination studies, either completed or in progress, will be reviewed in this presentation in terms of achieving complete disappearance of tumor, the durability of such remissions, and survival of patients so treated.

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REMISSION INDUCTION

The high complete response rate (81%) of patients treated with the MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) regimen between 1964 and 1967 (7) has been confirmed by a number of studies (*10, 11, 14*). Since 1967, only Stage IV-B patients have been treated with MOPP alone at the NCI. Despite this selection of only more advanced patients, the remission rate, in the 89 previously untreated Stage IV-B patients treated since July 1967, continues to be high, 76%. The ability to achieve remission is related to stage and performance status. It should be noted that, in the original NCI study, no patient with Stage III or IV-A disease failed to achieve a complete remission, and to date, only 1 has had a relapse (*7, 9*). Insertion of a significant number of asymptomatic patients into such a study will enhance the ability to achieve complete remissions and exert a favorable therapeutic bias. Designers of randomized trials should consider these observations in their initial patient stratification. The histopathologic presentation did not affect the ability to achieve a complete response in our study.

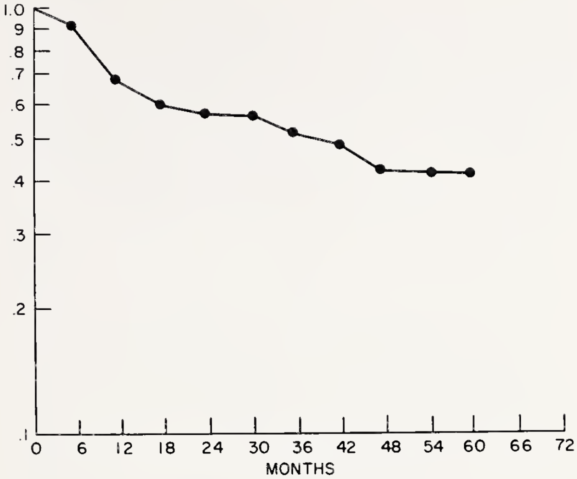
The study performed by Acute Leukemia Group B (ALGB) compared MOPP to a 5-drug combination (vincristine, procarbazine, vinblastine, chlorambucil, and prednisone) and to each of these drugs used singly in sequence (*14*). The complete remission rate with each combination was equivalent and proved superior to the same drugs used in sequence. Data are not yet available on the breakdown of the responses by the prognostic indications previously mentioned. A more recent study of ALGB compared 4 different drug combinations, 2 using 4 drugs [MOPP, and a similar program substituting 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) for nitrogen mustard], and 2 using only 3 drugs (BCNU, vincristine, and prednisone; and procarbazine, vincristine, and prednisone). The preliminary analysis of these data indicates that the 4-drug programs are superior in inducing complete remissions to those programs containing only 3 (*14*). In an uncontrolled trial by the Southwestern Cooperative Group (SWG), the complete remission rate in previously untreated patients using MOPP was 78% (*13*). The Southeastern Cooperative Group (SEG) performed a controlled trial comparing the remission induction of MOPP with nitrogen mustard. Although their complete remission rate with the drug combination (51%) was less than at the NCI and SWG, the differences between the rate of complete remissions with MOPP and nitrogen mustard (51 vs. 14%) are statistically significant (*10, 14*).

Other drug combinations have been tried in addition to those of the NCI and ALGB. The British elsewhere in this Symposium report similar results with a drug combination resembling MOPP which substitutes vinblastine for vincristine (*11*).

The Eastern Cooperative Oncology Group compared the combination of cyclophosphamide (10 mg/kg/wk), vinblastine (0.2 mg/kg/wk), and prednisone (1 mg/kg/day), to the same combination at one-third doses, and weekly vinblastine alone. The complete remission rate with the 3 treatments, 41, 14, and 10%, respectively, indicates the superiority of the full-dose combinations (*15*). Luce et al (*13*) have reported the results of an earlier SWG study using the same 3 drugs, cyclophosphamide, vincristine, and prednisone. The complete response rate in previously untreated patients (51%) was less than their subsequent experience with the MOPP regimen (*13*).

Certain types of prior treatment appear to influence the response to the drug combinations.

Data from 3 studies, NCI *(9, 16)*, British *(11)*, and SWG *(13)*, are available in enough detail to analyze these effects. All these studies confirm that prior radiotherapy does not compromise the patient's capability of responding in a satisfactory fashion to combinations of drugs. At NCI, a group of 21 patients who had had relapses after extensive radiation therapy (12 after total-nodal irradiation) were treated with MOPP from 2 to 49 months following X-irradiation (median, 19 months) *(9, 16)*. These patients achieved complete remission in 76% of cases, a result equivalent to the 75% achieved by the British group in the same type of patient *(11)*. The patients who had received major prior X-ray treatment in the SWG study also had a high rate of complete remissions, 71%. In contrast to these results, those patients who have had a significant prior chemotherapy, or a combination of chemotherapy and radiotherapy, in all 3 studies, had a significant reduction in the remission rate *(9, 16)*.



TEXT-FIGURE 1.—Life-table analysis of relapse rate in 35 patients with Hodgkin's disease who achieved a complete remission with MOPP between 1964 and 1967. *Ordinate* indicates fraction of patients continuously free of disease.

REMISSION DURATION

Data on the duration of remission following combination chemotherapy are available from 3 studies, NCI, SWG, and ALGB. The median duration of complete remission from the end of 6 cycles of MOPP at the NCI is 36 months *(8, 9)*. The relapse rate is greatest in the first 18 months and then declines progressively. Of most interest and importance is that, to date, we have not observed a relapse in any patient who, after the initial 6 cycles of treatment, remained free of disease in excess of 42 months. A life-table analysis of the relapse rate is illustrated in text-figure 1.

Since 1967, all patients with complete remission have been randomly allocated to 1 of 3 programs: no drug maintenance; intermittent treatment with MOPP (2 cycles at 3-month intervals); or BCNU given every 3 months, at a dose of 200 mg/m². At the present time, there are no significant differences among the 3 groups, though the numbers are insufficient to draw firm conclusions. The SWG randomly allocated complete responders to either no maintenance therapy or intermittent MOPP cycles (1 cycle every 2 months). Their long median duration of remission, 24 months, achieved without maintenance treatment, was prolonged to 36 months with intermittent cycles of MOPP *(13)*.

In a more recent study, this same cooperative group assigned randomized patients to maintenance therapy using only single drugs, in this case actinomycin D, methotrexate, or vinblastine. Preliminary results suggest no difference in the remission duration among the 3 drugs. In fact, the data from all 3 arms of the study are similar to the control arm (no maintenance therapy) of the previous study *(14)*.

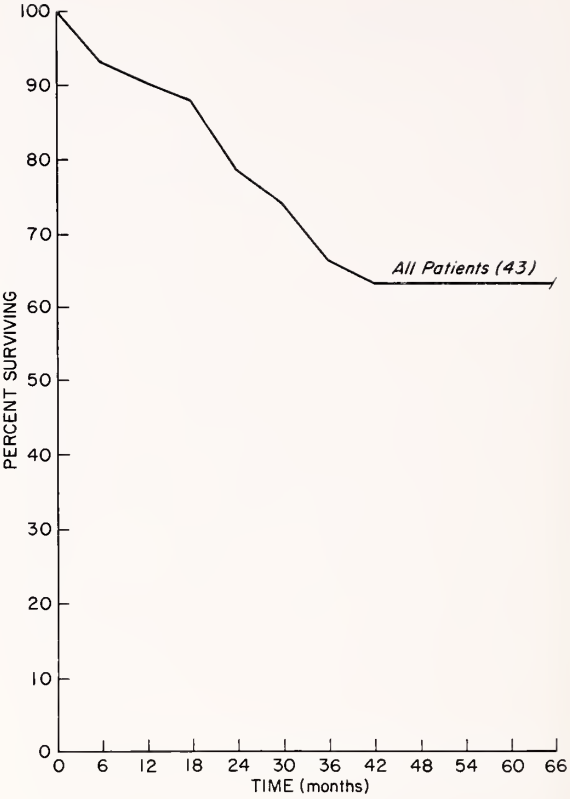
In the previously mentioned ALGB study, although the complete remission rate achieved with MOPP and the 5-drug combination was similar, the median remission duration after 6 cycles of MOPP was significantly superior to the ALGB 5-drug program *(14)*. In the ALGB's more recent study, patients achieving a complete remission after a variety of drug combinations are randomly allocated to maintenance treatment with 1 of 3 regimens, 2 employing only single agents: 1) daily chlorambucil alone; 2) daily chlorambucil and monthly reinduction courses of the drug combination; and 3) vinblastine alone, given weekly. In contrast to the SWG study, preliminary results indicate a prolongation of remission with all 3 forms of maintenance treatment, with a suggestion of superiority for the vinblastine arm (*14*).

SURVIVAL

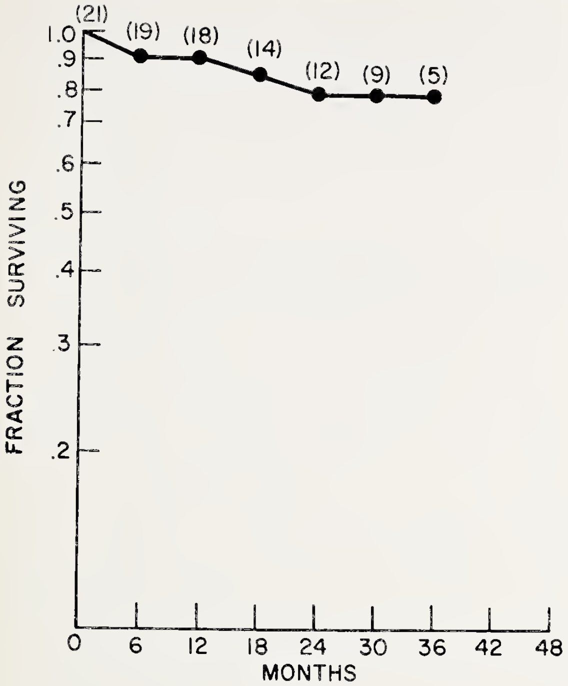
Data on controlled trials comparing survival rates with single agents to those with drug combinations are not yet available. Because of the longer follow-up time, only the published data from the NCI are available for a sufficient length of time and in significant detail to permit analyses (*8, 9*). These data show a marked improvement in survival of patients treated with MOPP over those treated in the past with single agents (*6, 8, 9*). The first group of 43 patients now has a minimum follow-up time just under 5 years, and a significant group of patients have now been followed up to 6 years from the end of all therapy. Of those patients who achieved a complete remission, 70% are alive at 6 years (*8, 9*). As illustrated in text-figure 2, the survival rate of the entire group of patients is >60% at 6 years. The median survival of the complete responders or the entire group has not been reached, but it will be >5 years. Of those patients who have had relapses, but are alive at 5 years, most are currently free of disease following retreatment with MOPP. We have noted that patients who relapsed after at least 6–12 months of remission appear to respond well to MOPP a second time, but require some form of intermittent exposure to the drugs to sustain the response. Survival was influenced by age. Those patients <30 years old fared significantly better than those 30–60 years old (*8*). Although the follow-up time is shorter, those patients treated at the NCI since 1967 have equivalent survivals at each point in time.

A preliminary analysis of the survival rate of 21 patients treated with MOPP following relapse after extensive X-irradiation is shown in text-figure 3 (*9*). Of the 16 complete responders, only 2 have had relapses, 1 of whom died 51 months after the onset of chemotherapy. Three others while in complete remission have died of second tumors. Autopsy in 2 of these patients revealed no evidence of Hodgkin's disease. This and other long-term complications of drug and drug plus X-irradiation therapy are described elsewhere in this Symposium. Eleven patients who achieved a complete remission remain alive and continuously free of disease. Of the 5 induction failures, 4 died at 1, 2, 12, and 19 months after the onset of chemotherapy, and 1 remains alive. The life-table analysis of the entire group (text-fig. 3) reveals that the surviving fraction for those at risk 2 years (12 patients) is 79%. Although the numbers are small, the survival is quite similar to the previously untreated group.

Analysis of the histopathologic data of all our patients reveals no relationship with survival at the present time (*7-9*). Likewise, within a given stage of the disease, anergy has not influenced response, remission duration, or survival (*17, 18*).



TEXT-FIGURE 2.—Life-table analysis of survival of patients contains all patients not just complete responders following MOPP treatment between 1964 and 1967.



TEXT-FIGURE 3.—Life-table analysis of the survival of 21 patients treated with MOPP following relapse after extensive X-irradiation. The *ordinate* indicates fraction of patients surviving. *Numbers in parentheses* indicate patients at risk for that time interval.

CONCLUSIONS

The use of drug combinations for the treatment of Hodgkin's disease appears superior to the use of the same agents given singly in conventional doses (*7–11, 13, 14*). The percentages of complete remissions achieved with all current drug combination programs are greater than those with single agents and usually are >50%. The complete remissions achieved are more durable, with median remission durations of 24–36 months. Survival is improved, and, most significantly, a certain proportion of patients remains continuously free of disease for periods of time up to 6 years after drug therapy is stopped (*8, 9*). These observations do not preclude the fact that the improved therapeutic effect noted with drug combinations is a result of the more aggressive approach inherent in the use of multiple drugs. No data are yet available to examine this question.

Several factors make it likely that the apparent prolongation in survival is not a spurious observation. A prolongation in survival usually occurs when the proportion of significant responses is >50%, and the duration of these responses is significantly prolonged. Indeed, a small fraction of patients responds favorably to single agents in standard doses (*6*), but, since the number never approached half of the population in question, the improved survival was often lost when only median survival data were examined. If the current durations of nondrug-maintained remissions achieved with combination chemotherapy are continuously confirmed, the coupling of a complete remission rate >50% with an approximately tenfold increase in remission duration must invariably result in prolonged survival.

There are several inconsistencies in the data of the several studies which may be explained by differences in the patient populations and protocol design. The original MOPP study was designed to give 6 cycles of treatment. It soon became apparent that, although many patients would respond promptly (median time to remission, 3 months), others, although continuing to respond, would still have residual disease after 6 cycles (6 months). Since 1967, we have modified our design to include continued treatment of such patients to complete remission, plus, if the patient had already received >6 cycles of therapy, at least 2 additional cycles. Some patients have required as many as 12 cycles of MOPP to achieve remission. Obviously, because of variations in the biologic characteristics of different patients, a fixed interval after which treatment is terminated will exclude slow responders from the responding group, particularly if the interval of treatment is as short as 6–8 weeks.

A difficulty exists in determining the endpoint for complete remission. Frequently the last parameter to return to normal is the lymphogram. Our criterion for normalcy in this test, post treatment, is a return of previously enlarged nodes to normal size. Architectural abnormalities rarely disappear on the early post-treatment films and may take years to return to normal on subsequently repeated studies. In our patients, biopsy of post-treatment lymph nodes has revealed extensive hyalinization, which would be expected to cause continued filling defects (*19*). Radiographers are understandably reluctant to consider such nodes technically normal, and it is advisable for all follow-up films to be reviewed by the physicians involved in the study. Variations in the interpretation of lymphograms can affect the complete remission rate or lead to a prolongation of the treatment period beyond that necessary to achieve remission.

The interval between cycles of treatment varies between studies and is affected by the physician's response to the observed toxicity. Some physicians prefer to wait until all blood counts have returned to normal before instituting the next cycle. At the NCI, we have never observed a complete remission in patients whose tumor regrew between cycles. This has influenced our study design. Using a sliding scale, we prefer to institute therapy at lower doses rather than risk tumor regrowth by prolonging the intervals between cycles. The influence of prolonging the intervals between cycles will obviously also vary with the biologic aggressiveness of a patient's tumor. Short periods of therapy with a fixed time limit tend to exclude slow responders from the good response group, while long intervals between cycles do the same for those patients with more aggressively growing tumors.

Although no significant effect of the histopathologic presentation on survival has been noted to date, it is likely that such an effect will eventually be demonstrated (*7–9*). For example, patients with nodular sclerosis (NS) and Stage IV Hodgkin's disease at the NCI had remission rates equal to other histopathologic subgroups. However, the remission duration and fraction remaining free of disease in this subgroup are less than those with the mixed-cellularity or lymphocyte-depleted subgroups (*9*). It seems likely that the favorable natural history of the NS form of the disease has influenced the present mortality rates. Since many of the patients with NS Hodgkin's disease have had relapses at least once, they can be expected to ultimately die of Hodgkin's disease earlier than those who have been continuously free of disease.

At the moment, remission durations achieved with MOPP appear superior to other reported studies; thus, although the original 5-drug ALGB combination achieved a similar response rate, the durations of subsequent remissions with MOPP are reported to be superior (*14*). The difference not only suggests a superiority of MOPP in reduction of tumor volume, but also illustrates that the endpoint of "complete remission" cannot be used alone to determine the efficacy of a particular drug program.

The difference in the ability to maintain remissions with single agents between the SWG and ALGB studies previously noted suggests a significant difference in patient mix or criteria for complete remission. Thus, ALGB appears to be showing a prolongation of remission with single agents with a trend toward superiority for vinblastine, while the SWG shows no prolongation of remission with any of the single drugs employed, including vinblastine (*14*). Neither group study currently includes a control arm with no maintenance therapy. A true test of the patient mix is therefore not occurring. A possible reason for the discrepancy, related to the histopathology of the patient population, is suggested by the results of the European Cooperative Group. Employing vinblastine or no drug therapy after X-irradiation for various stages, this group has observed a significant advantage of vinblastine only in those patients with lymphocyte-depleted histology within a given stage (*20*).

Although the treatment of the advanced stages of Hodgkin's disease has improved with the use of drug combinations, a significant fraction of patients fails to achieve a remission, and at least half of those who do can be expected to have relapses within 5 years. The goals of future chemotherapy trials should be the devising of additional combination regimens which differ significantly in drug content from present ones. Such combinations should be compared in a controlled fashion with the currently effective treatments, and the duration of non-drug-maintained remission compared as an additional control on the therapeutic efficacy of each new study. Historical controls of remission duration are clearly insufficient. Two different and effective combination regimens might reasonably be employed in tandem to prevent recurrences. Uniform criteria for evaluation of response and more attention to stratification of patients according to parameters known to influence prognosis would insure a greater validity of the data and facilitate the interpretations *(21)*.

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Morbus Hodgkin's Disease, Stages I and II: Trial of the European Organization for Research on Treatment of Cancer[1]

B. van der Werf-Messing,[2] *Rotterdam Radiotherapy Institute, Rotterdam, The Netherlands*

SUMMARY—Between 1964 and 1971, 296 patients with early Hodgkin's disease were admitted to a cooperative trial, conducted by Cancer Centers in France, Belgium, and Holland. The patients were randomized in two treatment modalities: *a*) Irradiation to lymph nodes at one side of the diaphragm (mantle technique or inverted Y technique). *b*) The same course of irradiation followed by vinblastine treatment once a week for 2 years. The actuarial survival rate and the relapse-free survival were more favorable after additional vinblastine therapy. Vinblastine appears most effective in the histologic subgroup "mixed cellularity." The preliminary results of this trial have been utilized for the design of a new European Cooperative Trial in early Hodgkin's disease.—Natl Cancer Inst Monogr 36: 331–386, 1973.

IN 1964 the cooperative group of radiotherapists and chemotherapists of the European Organization for Research on Treatment of Cancer (E.O.R.T.C.) started a combined trial in early Hodgkin's disease to assess whether, in Stages I and II, A and B (Rye classification), additional chemotherapy after extended radiotherapy could improve prognosis. Furthermore this group hoped that, by meticulously recording data, they would accumulate a body of knowledge that might lead to therapeutic options and further improvement of prognosis.

All patients were staged clinically without laparotomy. The histology of all the cases was reviewed by an European panel of pathologists. All lymphographies were assessed by a group of radiodiagnostic experts. The radiotherapy techniques and physical irradiation data were checked by physicists of the study group.

The trial protocol was as follows: Patients with Hodgkin's disease Stages I and II, A and B, received radiation to the involved side of the diaphragm only; 4000 rads in 4 weeks was given to these areas. The prophylactic regions on the same side of the diaphragm received 3500 rads in the same period. Six weeks after radiation treatment, the patients were reassessed and, when there was no evidence of disease, they were placed at random into two groups. Group I received no further treatment and group 2, chemotherapy. Chemotherapy consisted of intravenous injection of vinblastine once weekly for 2 years (>5,000 leukocytes, 10 mg; leukocytes >3,000–<5,000, 5 mg; leukocytes <3,000, no vinblastine).

In 1971, admission into the trial stopped after 296 patients had been included. Table 1 presents the main characteristics of the trial cases. The 5-year survival of all patients was 75%. Additional chemotherapy did not significantly influence survival (text-fig. 1).

Disease-free survival was significantly better with additional chemotherapy during the first 2 years (text-fig. 2). Text-figure 3 demonstrates survival according to the histologic type. Prognosis was significantly better for patients having LP and NS than in those with MC and LD. The disease-free survival showed the same pat-

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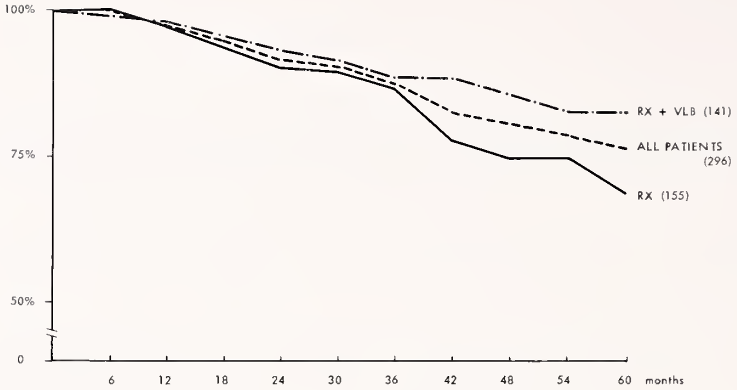
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] President of European Organization for Research on Treatment of Cancer. Presented on behalf of all cooperating centers.



TEXT-FIGURE 1.—Actuarial survival. RX = X-ray; VLB = vinblastine.

TABLE 1.—*E.O.R.T.C. trial: Hodgkin's disease, Stages I and II, A and B*

Total No. of patients	296
Male	183
Female	113
A	257
B	39
I	216
II	80
Above diaphragm	282
Below diaphragm	14
LP*	35
NS†	95
MC‡	99
LD§	8

*Not all cases have yet been re-evaluated histologically. LP = lymphocyte predominance.
†Nodular sclerosis.
‡Mixed cellularity.
§Lymphocyte depletion.

tern (text-fig. 4). In the case of LP, additional chemotherapy had no demonstrable influence on either the survival rate or the disease-free survival (text-figs. 5, 6). In the histologic group, NS survival was not influenced by the type of treatment given, though the group that received additional chemotherapy had a slightly better remission-free survival (text-figs. 7, 8).

In the MC group, 5-year survival after X-ray therapy alone was about 50%, whereas additional chemotherapy increased survival to 75%. This beneficial influence of additional vinblastine is also evident up to 2 years if disease-free survival rates are compared between the 2 treatment modalities. After 2 years, the disease-free survival became similar in both treatment groups (text-figs. 9, 10).

The small number in the LD group did not allow analysis of the subgroups.

From the preliminary data of this trial, it can be concluded that chemotherapy improves prognosis significantly in the histologic subgroup, MC. Also the high incidence of relapses, most of which occurred beyond the irradiated area (table 2), suggests that irradiation of the subdiaphragmatic region can improve prognosis.

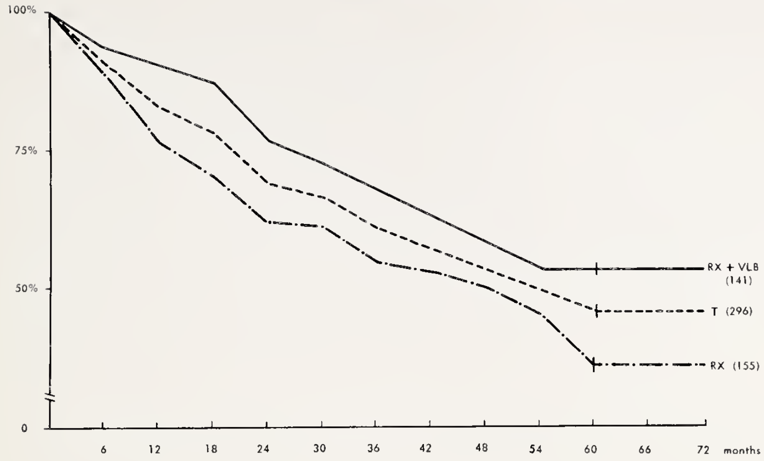
TABLE 2.—*E.O.R.T.C. trial: Type of recurrence versus type of treatment**

	X-ray	X-ray +	Total
Local recurrence (in irradiated area)	6	5	11
Extension beyond diaphragm	31	14	45
Visceral or disseminated disease	21	19	40
Total	58	38	96

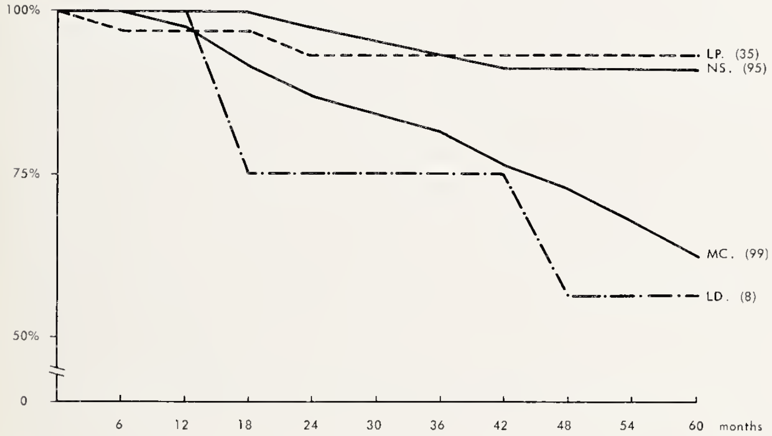
*Data to 1/1/72.

From these preliminary conclusions, the protocol was designed for a new trial for patients with Hodgkin's disease Stages I and II, A and B (text-fig. 11).

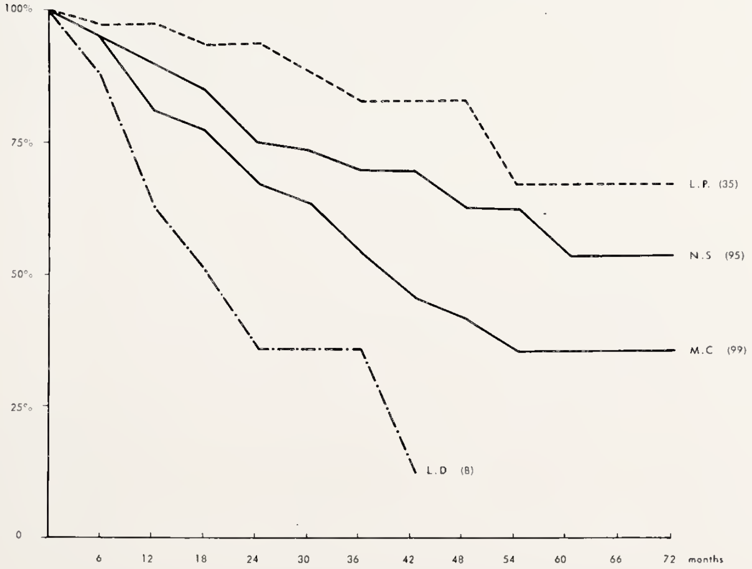
All follow-up data of this trial will continuously be meticulously recorded. They will enable us to identify in the future those groups having a good prognosis with conservative



TEXT-FIGURE 2.—Actuarial relapse-free survival. RX = X-ray; VLB = vinblastine; T = total.



TEXT-FIGURE 3.—Actuarial survival according to histology.

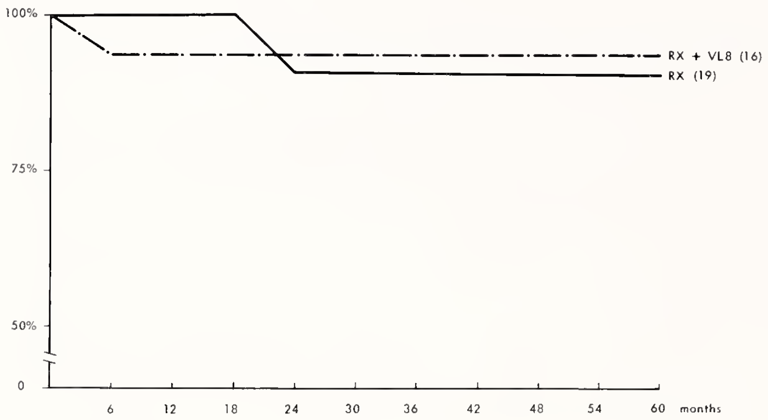


TEXT-FIGURE 4.—Actuarial relapse-free survival according to histology.

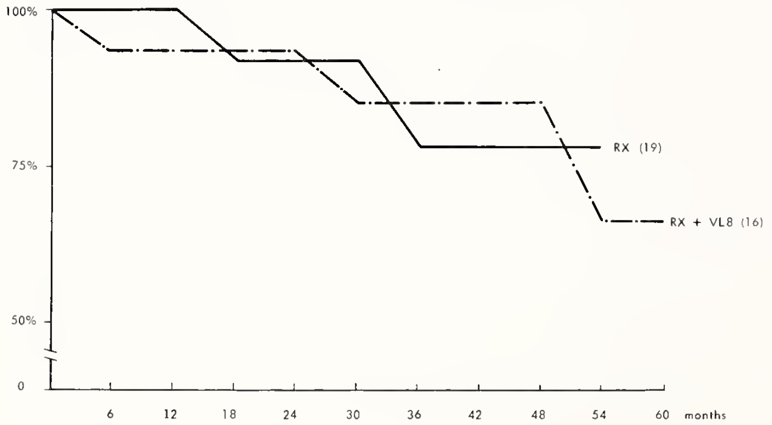
treatment and those in which extensive radical treatment is justified to offer the best possible chance of cure.

The Chairmen of the Cooperative Group of Radiotherapists and Chemotherapists have been: M. Tubiana (1964–67), K. Breur (1967–69), and B. van der Werf-Messing (1969–72). The Secretary-General is A. Laugier. The statisticians are: M. Hayat and H. Amar. Members of the Committee on Histology are: R. Gérard Marchant and J. A. M. van Unnik. Members of the Committee on Lymphography are: R. W. Kropholler, J. D. Picard, P. Markovits, and J. L. Chassard.

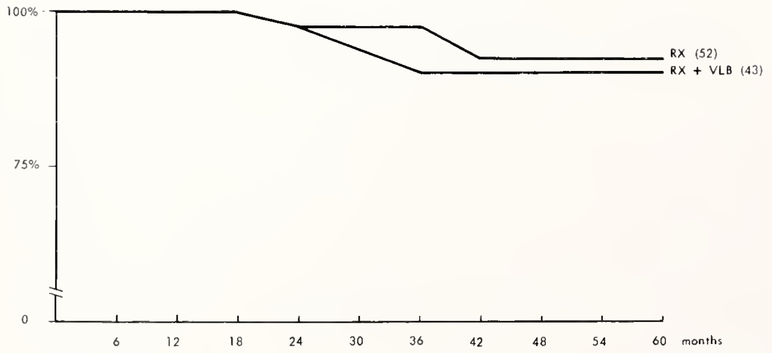
Cooperative Centers have been: Rotterdamsch Radio-Therapeutisch Instituut, Rotterdam, The Netherlands; Institut Gustave Roussy, Villejuif, France; Antoni van Leeuwenhoekhuis, Amsterdam, The Netherlands; Academisch Ziekenhuis, Leyden, The Netherlands; Hôtel Dieu, Paris, France; Centre Anti Cancéreux, Caen, France; Institut Jules Bordet, Brussels, Belgium; Centre Léon Bérard and Hôpital Edouard Herriot, Lyon, France; Hôpital de Bavière, Liege, Belgium; St. Radboud Ziekenhuis, Nimeguen, The Netherlands; Fondation Bergonié, Bordeaux, France; Centre Anti Cancéreux, Rouen, France; Gemeenteziekenhuis Zuidwal, The Hague, The Netherlands; and Centre Anti Cancéreux, Louvain, Belgium.



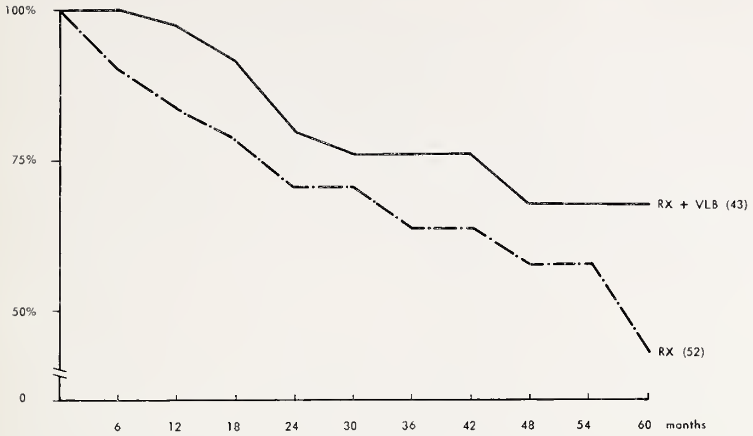
TEXT-FIGURE 5.—Actuarial survival according to vinblastine (LP). RX=X-ray; VLB=vinblastine.



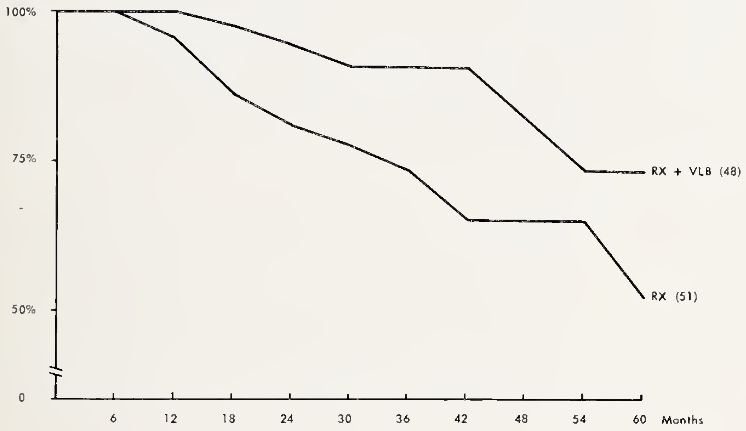
TEXT-FIGURE 6.—Actuarial relapse-free survival according to vinblastine (LP). RX=X-ray; VLB=vinblastine.



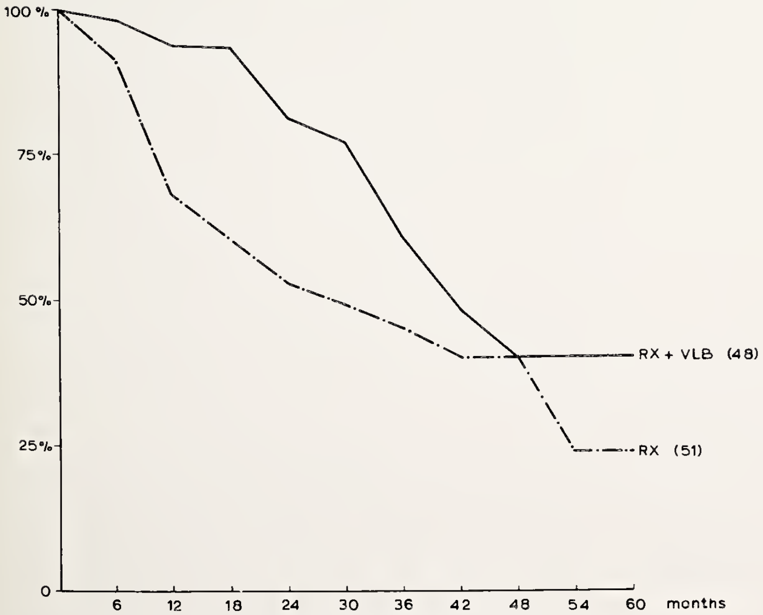
TEXT-FIGURE 7.—Actuarial survival according to vinblastine (NS). RX=X-ray; VLB=vinblastine.



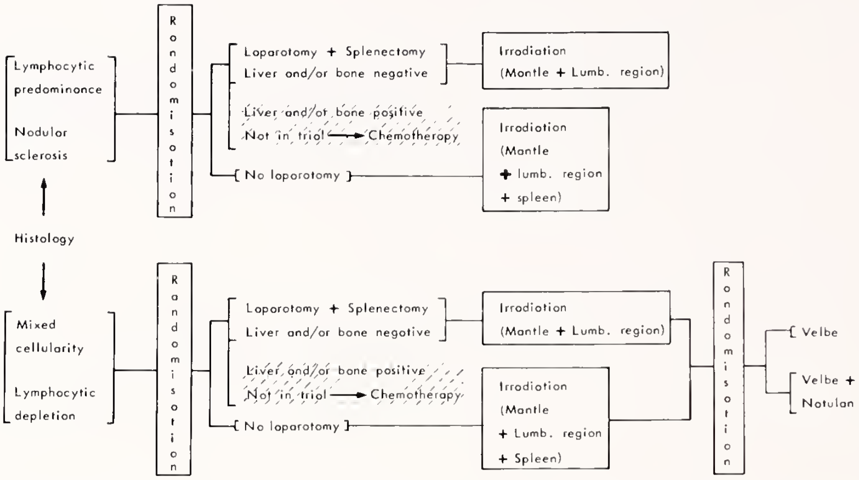
TEXT-FIGURE 8.—Actuarial relapse-free survival according to vinblastine (NS). RX=X-ray; VLB=vinblastine.



TEXT-FIGURE 9.—Actuarial survival according to vinblastine (MC). RX=X-ray; VLB=vinblastine.



TEXT-FIGURE 10.—Actuarial relapse-free survival according to vinblastine (MC). RX=X-ray; VLB=vinblastine.


Lymphocytic predominance
Nodular sclerosis
Histology
Randomisation
Laparotomy + Splenectomy
Liver and/or bone negative
Irradiation
(Mantle + Lumb. region)
Liver and/or bone positive
Not in trial → Chemotherapy
No laparotomy
Irradiation
(Mantle
+ lumb. region
+ spleen)
Mixed cellularity
Lymphocytic depletion
Randomisation
Laparotomy + Splenectomy
Liver and/or bone negative
Irradiation
(Mantle + Lumb. region)
Liver and/or bone positive
Not in trial → Chemotherapy
No laparotomy
Irradiation
(Mantle
+ Lumb. region
+ Spleen)
Randomisation
Velbe
Velbe + Natulan

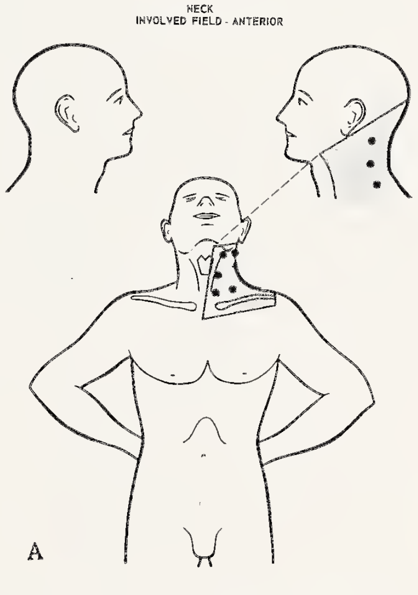
Text-figure 11.—New trial for patients with Hodgkin's disease, Stages I and II, A and B.

Progress Report. Hodgkin's Clinical Trial, 1972 [1,2]

George B. Hutchison, *Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts 02115*

SUMMARY—The 5th year of a collaborative clinical trial to evaluate 2 regimens of radiotherapy has just ended; 383 patients were enrolled. Patients similar in clinical stage, age, and histologic classification distributions were assigned to both regimens. Patients with nodular-sclerosing histologic type Hodgkin's disease were found initially to have contiguous anatomic type disease patterns, whereas patients with mixed-cellularity type Hodgkin's disease showed no more than a chance tendency to these patterns. Follow-up study showed a greater tendency to local extension in patients treated with involved-field regimens, but we found no such difference for major extensions. There was no difference in survival rate during the first 3 years after treatment.—Natl Cancer Inst Monogr 36: 387–393, 1973.

IN MARCH, 1967, a collaborative clinical trial was undertaken to evaluate the relative effectiveness of 2 regimens of radiotherapy for localized Hodgkin's disease, Rye Stages I and II. The regimens will be referred to as involved-field (IF) and extended-field (EF) therapy. The IF regimen is intended to deliver a tumoricidal dose to all known areas of Hodgkin's disease involvement without irradiating tissue not known to be involved. The EF regimen considers the known orderly progression of Hodgkin's disease and directs radiotherapy to the areas of known involvement and areas with a high probability of future involvement. For example, a patient with known involvement of nodes only in the left neck would receive treatment only to the left neck (text-figs. 1A , 1B) under the IF regimen. Under the EF regimen, however, he would be treated in the entire neck, both axillae, the mediastinum, upper para-aortic nodes, and the spleen (text-figs. 2A, 2B). This description of the EF regimen was adopted by most of the collaborating centers in 1969 and 1970. Before that, concern about possible complications of EF's encouraged treatment of a somewhat more restricted EF, with para-aortic areas and spleen omitted in patients with disease confined to the neck. A manual of treatment fields has been



TEXT-FIGURE 1.—*A*) Neck, IF, anterior.

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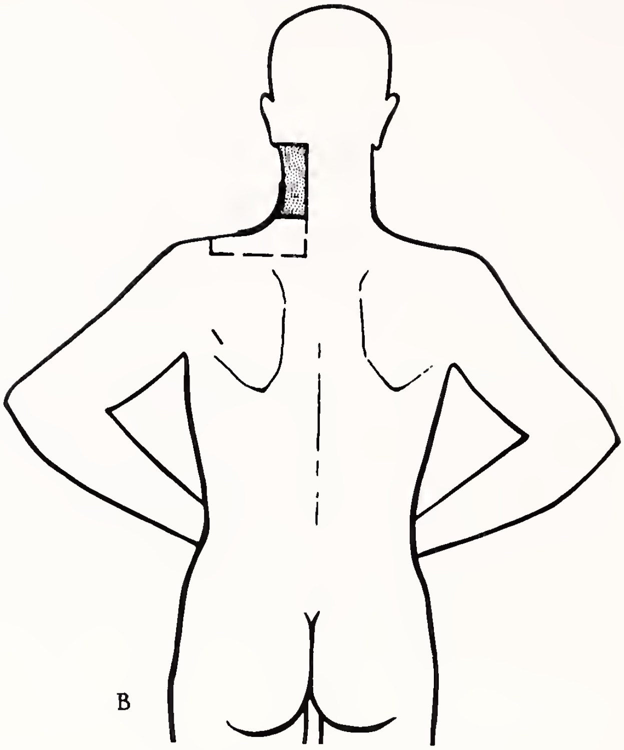
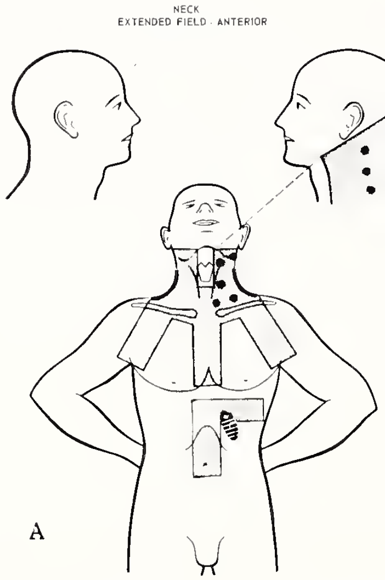
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported in part by Public Health Service grants CA8970, CA8971, and CA13607 from the National Cancer Institute.



Text-figure 1.—*B)* Neck, IF, posterior.



Text-figure 2.—*A)* Neck, EF, anterior.

prepared illustrating appropriate fields for all common distributions of disease. A dose of at least 3500 R in 3–4 weeks is given to all prescribed fields.

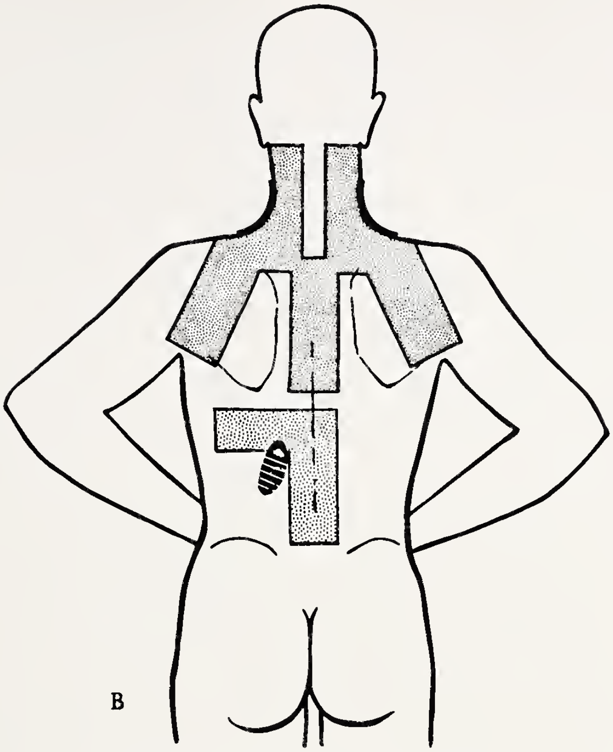
Approximately 470 eligible patients must be enrolled, 235 in each regimen, to detect a survival benefit for EF therapy large enough to be of clinical interest.

Table 1 indicates the current enrollment. Twenty-two centers have contributed 383 active patients of the planned 470. The 4 centers which contributed the most patients are Princess Margaret Hospital, Walter Reed Hospital, M. D. Anderson Hospital, and Memorial Hospital.

Case accession has been about 100 per year since 1967.

INITIAL FINDINGS

The next several tables show this patient population by stage, age, and histology. Most are Stage II patients, with $\geqq 3$ regions involved or with 2 nonadjacent regions involved. Stage I-1 is next most common, and Stage I-2, with 2 adjacent regions affected, is least common. This breakdown by stage is similar for both treatment groups (table 2).



Text-figure 2.—*B)* Neck, EF, posterior.

Table 1.—*Enrollment by clinic*

Clinic	Patients		
	Active	Excluded	Total
University of Wisconsin Medical Center	24	7	31
Princess Margaret Hospital	59	9	68
Walter Reed	42	8	50
M. D. Anderson Hospital and Tumor Institute	60	3	63
Michael Reese Hospital	13	2	15
Medical College of Virginia	3	3	6
University of Oregon Medical School	14	7	21
Allegheny General Hospital	18	2	20
University of Pennsylvania Hospital	2	0	2
University of Chicago Hospitals	26	5	31
Veterans Administration Hospitals	8	1	9
University of Iowa Hospital	1	2	3
University of Minnesota Hospital	1	1	2
Vanderbilt University Hospital	14	0	14
New York University Hospital	0	1	1
Montefiore Hospital and Medical Center	2	1	3
Mt. Sinai Hospital	12	1	13
University of Washington Hospital	11	2	13
Memorial Hospital for Cancer and Allied Diseases	62	7	69
City of Hope National Medical Center	10	1	11
The Children's Orthopedic Hospital and Medical Center	0	1	1
Children's Hospital of Columbus	1	1	2
Total	383	65	448

TABLE 2.—*Stage by treatment*

Stage	IF	EF	Total
I-1	47	65	112
I-2	44	46	90
II	81	76	157
Unknown	11	13	24
Total	183	200	383

These are young people, with modal decade of birth 1940–49. The frequency diminishes as one moves to older or younger age groups, and we have only 11 patients born before 1910. Again there is no significant age difference between IF and EF groups (table 3).

TABLE 3.—*Year of birth by treatment*

	IF	EF	Total
Before 1910	4	7	11
1910–19	15	10	25
1920–29	13	21	34
1930–39	27	39	66
1940–49	70	82	152
1950 or later	43	32	75
Unknown	11	9	20
Total	183	200	383

Two histologic categories in the Rye classification account for the overwhelming majority of patients—306 of 348 for whom slides have been received. About 200 patients have nodular-sclerosing (NS), and 100 have mixed-cellularity (MC) type. These slides were classified at the pathology center by Dr. H. Rappaport and his staff (table 4).

TABLE 4.—*Pathology category, Rye classification by pathologist of record, by treatment*

	IF	EF	Total
Lymphocytic predominance	13	17	30
NS	94	104	198
MC	49	59	108
Lymphocytic depletion	4	2	6
Unclassifiable*	5	1	6
Unknown†	18	17	35
Total	183	200	383

*Diagnosis of Hodgkin's disease confirmed, but histologic subtype cannot be determined.

†Slides not yet reviewed by pathologist of record.

The initial evaluation of patients includes routine history, routine physical examination, lymphogram, intravenous pyelogram, and liver function evaluation, as well as biopsy.

After he makes his evaluation, the participating investigator notifies the coordinating center and is given a treatment assignment. Enrollment, initial evaluation, treatment, and follow-up are reported to the coordinating center on standard forms. Sixty-five patients were were given. These were patients for whom the diagnosis of Hodgkin's disease was not confirmed, whose stage was not localized, or who failed to satisfy some other study criterion.

One of our earliest investigations (*1*) of this patient material concerned the anatomic disease pattern at diagnosis. This was of interest because of the underlying thesis of an orderly progression of disease. Two concepts of orderly progression were compared. First we supposed that disease might tend to spread to adjacent sites. If this were so, spread from left neck to left axilla would be common, for example, while spread from left neck to right axilla would be rare. Second, the disease might extend from initial sites to the most common available site, whether adjacent or not. If this occurred, extension from left neck to right neck would be the most common pattern and spread to right axilla would have the same frequency as spread to left axilla.

Table 5 shows that, when 2 regions are involved, an ipsilateral neck-axilla pattern is much more common than a contralateral pattern. This suggests that the initial site does affect the location of the second site. Surprisingly, however, the combination of the 2 sides of the neck is much less common than neck-axilla or neck-mediastinum. By chance, bilateral neck would be most common, since the 2 sides of the neck alone are the 2 most common sites for disease confined to 1 site. This is interpreted to mean that spread tends toward contiguous sites, along normal lymphatic channels, rather than toward adjacent sites.

Table 6 investigates this same relationship separately for the 2 common histologies, NS and MC. Whether adjacent or contiguous spread is considered, NS closely adheres to the orderly pattern. MC, on the other hand, shows no more

TABLE 5.—*Groups of 2 anatomic regions*

Regions	Number of patients		Proportion	
	Observed	Expected	Observed	Expected
Bilateral neck	7	19.4	0.16	0.441
Ipsilateral neck, axilla	13	7.3	.30	.166
Contralateral neck, axilla	1	7.2	.02	.163
Neck, mediastinum	21	8.1	.48	.183
Bilateral axillae	2	0.6	.05	.014
Axilla, mediastinum	0	1.4	.00	.032
Total	44	44.0	1.01	.999

TABLE 6.—*Observed and expected numbers, by histologic type: Multiple regions involved*

	Number of patients			
	NS		MC	
	Observed	Expected	Observed	Expected
Groups of 2 regions:				
Bilateral neck	2	10.1	3	5.3
Ipsilateral neck, axilla	7	3.8	4	2.0
Contralateral neck, axilla	1	3.8	0	2.0
Neck, mediastinum	13	4.2	4	2.2
Bilateral axillae	0	0.3	1	0.2
Axilla, mediastinum	0	0.7	0	0.4
Groups of 3 regions:				
Bilateral neck, axilla	0	10.2	1	5.0
Bilateral neck, mediastinum	17	5.7	4	2.8
Neck, bilateral axillae	0	1.2	4	0.7
Ipsilateral neck, axilla, mediastinum	3	1.4	0	0.7
Contralateral neck, axilla, mediastinum	0	1.4	0	0.7
Bilateral axillae, mediastinum	0	0.1	1	0.06
Groups of 4 regions:				
Bilateral neck, axillae	0	1.7	1	1.1
Bilateral neck, axilla, mediastinum	5	4.0	0	2.6
Neck, bilateral axillae, mediastinum	1	0.3	3	0.2
Total	49	48.9	26	25.96
Adjacent regions	47	41.1	17	21.7
Nonadjacent regions	2	7.8	9	4.3
Total	49	48.9	26	26.0
Contiguous regions	45	19.1	12	10.3
Discontiguous regions	4	29.8	14	15.7
Total	49	48.9	26	26.0

than a chance likelihood to either adjacent or contiguous spread.

FOLLOW-UP

Table 7 shows preliminary follow-up data. No patients have yet reached 5 years, and only 10 patients have been observed at 4 years. We have enough observations at 3 years to permit some confidence that survival up to that point does not differ between the 2 treatment regimens. The 3-year survivors were all enrolled under the old definition of EF, with abdominal nodes treated only if the mediastinum was involved. In recent years, EF patients at most centers have been treated in upper abdominal nodes, and survival data are available for these patients up to 2 years. There is no difference in survival between the 2 treatments for these new series up to 2 years. Neither is there any difference between survival of the old and the new series patients in these early years.

We are comparing the 2 treatments in another way. Table 8 presents extension-free survival. The 2 left columns show that 58 IF patients have extensions as compared with only 37 EF patients. The extension-free survival table shows an early separation of the 2 series. This difference, however, must be interpreted carefully. This is not a double-blind study, and particularly in the early months, during treatment of IF's, the investigator may be alert to marginal extension and report and treat it promptly. Extension in the same regions already under treatment or planned for treatment may be unnoticed or not reported when EF therapy has been assigned.

The 2 right columns show investigation of this possible bias. We considered only extensions to distant sites—across the diaphragm or to extranodal organs. There are 41 distant extensions of patients in the IF group and 37 in the EF group, which is acceptable as a chance fluctuation.

TABLE 7.—*Survival*

Months	Number at risk		Percent survival		Number of deaths in interval	
	IF	EF	IF	EF	IF	EF
0	183	200	100	100	2	3
6	146	168	99	98	1	2
12	126	135	98	97	4	1
18	100	101	94	96	1	1
24	72	77	93	94	5	3
30	55	51	86	90	1	2
36	35	29	84	86	2	0
42	24	15	—	—	2	0
48	6	4	—	—	1	0
54	—	1	—	—	—	0
		Total No. of deaths			19	12

ACKNOWLEDGMENTS

Data presented in this paper were obtained from the following institutions: University of Wisconsin Medical Center, Madison, Wisconsin (William L. Caldwell, M.D.); Princess Margaret Hospital, Toronto, Canada (Ruth Alison, M.D.); Walter Reed General Hospital, Washington, D.C. (James D. Cox, M.D.); M. D. Anderson Hospital and Tumor Institute, Houston, Texas (Lillian M. Fuller, M.D.); Michael Reese Hospital, Chicago, Illinois (James J. Nickson, M.D.); Medical College of Virginia, Richmond, Virginia (E. Richard King, M.D.); University of Oregon Medical School, Portland, Oregon (Clifford V. Allen, M.D.); Allegheny General Hospital, Pittsburgh, Pennsylvania (John C. Frich, Jr., M.D.); University of Pennsylvania Hospital, Philadelphia, Pennsylvania (Walter L. Eaton, Jr., M.D.); University of Chicago Hospitals, Chicago, Illinois (John E. Ultmann, M.D.); Veterans Administration Hospitals, Cleveland, Ohio (Prentiss M. Dettmann, M.D.); Veterans Administration Hospital, Iowa

TABLE 8.—*Extension-free survival, percent*

Months	All extensions		Distant extensions	
	IF	EF	IF	EF
0	100	100	100	100
6	88	95	94	95
12	77	86	86	86
18	64	79	75	80
24	56	76	68	76
30	52	71	63	71
36	47	66	59	66
Population at risk	183	200	183	200
Extensions	58	37	41	37
Deaths without extension	4	2	5	2

City, Iowa (David T. Kaung, M.D.); University of Iowa Hospital, Iowa City, Iowa (Howard B. Latourette, M.D.); University of Minnesota Hospital, Minneapolis, Minnesota (K. K. N. Charyulu, M.D.); Vanderbilt University Hospital, Nashville, Tennessee (Silvio A. Aristizabal, M.D.); New York University Hospital, New York, New York (Joseph Newall, M.D.); Montefiore Hospital and Medical Center, New York, New York (George Schwarz, M.D.); Mt. Sinai Hospital of New York, New York, New York (Arvin S. Glicksman, M.D.); University of Washington Hospital, Seattle, Washington (Arthur J. Gerdes, M.D.); Memorial Hospital for Cancer and Allied Diseases, New York, New York (Burton J. Lee, M.D.); City of Hope National Medical Center, Duarte, California (Melville L. Jacobs, M.D.); The Children's Orthopedic Hospital and Medical Center, Seattle, Washington (J. Trenholme Griffin, M.D.); and Children's Hospital of Columbus, Columbus, Ohio (William A. Newton, Jr., M.D.).

We are greatly indebted to the radiotherapists, pathologists, and internists at the above institutions whose contributed work made this study possible. We also thank Dr. Henry Rappaport, University of Chicago, Chicago, Illinois, who reviewed pathology sections from all collaborating institutions; Dr. Manuel Viamonte, University of Miami, Miami, Florida, who reviewed lymphangiogram films from all collaborating institutions; and other members of the steering committee, Dr. James J. Nickson, Dr. Henry S. Kaplan, Dr. Saul Rosenberg, Dr. Burton J. Lee, Dr. Lillian M. Fuller, Dr. M. Vera Peters, and Dr. Robert J. Shalek, who gave continued guidance during the execution of the study.

REFERENCE

(*1*) HUTCHISON GB: Anatomic patterns by histologic type of localized Hodgkin's disease of the upper torso. Lymphology 5:1–14, 1972

Combination Chemotherapy in Advanced and Recurrent Hodgkin's Disease [1]

T. J. McElwain,[2] P. F. M. Wrigley, Alice Hunter, D. Crowther, J. S. Malpas, Michael J. Peckham,[2] D. W. Smithers, *and* **G. Hamilton Fairley,[3]** ***Institute of Cancer Research, The Royal Marsden Hospital, Sutton, Surrey, England; and the Department of Medical Oncology, St. Bartholomew's Hospital, London, England***

SUMMARY—One hundred and seventy-five patients with advanced or recurrent Hodgkin's disease were treated with a combination of nitrogen mustard, vinblastine, procarbazine, and prednisone (MVPP). Complete remission rate was 76.6%, and the predicted survival rate for the group is 59.2% at 3 years. The results agree closely with those obtained with the original MOPP combination (nitrogen mustard, vincristine, procarbazine, and prednisone) over which MVPP may have advantages in selected patients. All patients received maintenance treatment after the first 6 cycles of treatment, but, to date, this does not seem to have produced significant benefit when compared with the group of patients treated with MOPP whose treatment was discontinued after the initial induction phase.—Natl Cancer Inst Monogr 36: 395–402, 1973.

SINCE THE introduction of the MOPP regimen (a combination of nitrogen mustard, vincristine, procarbazine, and prednisone) by DeVita, Serpick, and Carbone (*1, 2*), high-dose, intermittent, combination chemotherapy has become the treatment of choice for patients with widespread Hodgkin's disease. We have been using a modification of the MOPP regimen since 1968 and have reported our early experience with 52 patients (*3*) and with 140 patients (*4*). We have now treated nearly 200 patients, and this paper reports our experience with 175 of these patients who can be completely evaluated in terms of histology, stage of disease, and follow-up.

PATIENTS AND METHODS

Treatment.—Details of the combination (MVPP) are shown below.

	First 6 courses
Nitrogen mustard	6 mg/m^2 intravenously daily on days 1 and 8
Vinblastine	6 mg/m^2 intravenously on days 1, 8, and 14
Procarbazine	100 mg/m^2 orally daily on days 1–14
Prednisone	40 mg orally daily on days 1–14

The combination differs from MOPP as follows: *a*) Vinblastine is used in place of vincristine, and a third dose is given on day 14; *b*) prednisone is given during every course of treatment. A maximum daily dose of 40 mg per adult patient is never exceeded; *c*) a rest period of

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] We gratefully acknowledge the financial help from the Buchanan Trust, which enabled us to attend this Symposium.

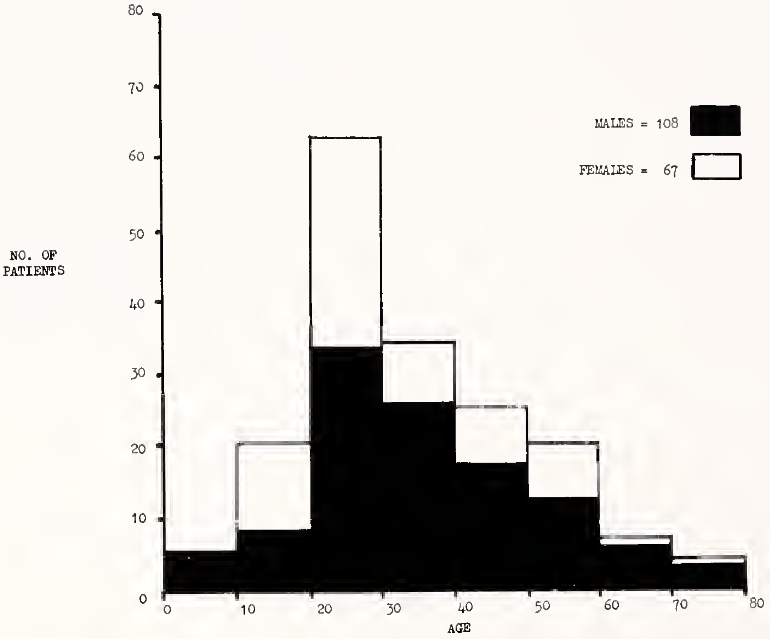
[3] We are grateful to the many physicians, surgeons, and radiotherapists who have referred patients to us. Histologic grading was carried out by Dr. Iris Hamlin, Professor Noel Gowing, and Dr. A. G. Stansfeld. Most of the lymphograms were reported by Dr. J. S. Macdonald and Dr. I. Kelsey Fry, and the isotope scans were done by Dr. V. R. McCready and Miss Joan MacAllister. Mrs. Elizabeth Austin and Mrs. Yvonne Terry and their staffs collected the data upon which this paper is based. Statistical assistance was provided by Mr. Peter Payne and Mr. A. Skeet. To all these people we offer our sincere thanks.

4 weeks is left between the end of one course and the start of the next course; and *d*) after 6 courses, all patients receive maintenance chemotherapy. This consists of the same drugs in the same doses but differs from the first 6 courses in that the interval between courses is increased.

Maintenance.—Full reassessment is done 3 months after Course 6: If patient is in complete remission, he is given 4 courses at 3-month intervals; 3 courses at 4-month intervals; and finally 3 courses at 6-month intervals. Chemotherapy is then discontinued.

If significant bone marrow toxicity develops, dosage modifications are left to the judgment of the physician. However, our general policy has been to modify the vinblastine dosage before altering the doses of the other drugs. This has most commonly meant that the day-14 dose of vinblastine has been omitted, and this will be discussed in more detail later. Most patients have been admitted to hospital overnight for injections of nitrogen mustard (days 1 and 8) because they require sedation and antiemetics to reduce the nausea produced by the drug. Some patients have been prepared to tolerate this side effect without sedation, and these have received their injections as outpatients.

Patients studied.—There were 175 patients, 108 males and 67 females ranging in age from 8 to 76 years; follow-up times ranged from 2 months to 4 years. A total of 122 patients have been followed for 1 year, 59 for 2 years, 18 for 3 years, and 1 for 4 years. The age and sex distribution is shown in text-figure 1.



Text-figure 1.—Age and sex distribution of 175 patients.

Histology.—Histologic classification was by the criteria of Lukes and Butler (*5*). A total of 8 patients (4.6%) had lymphocyte predominant (LP), 91 (52.0%) had nodular sclerosis (NS), 51 (29.1%) had mixed cellularity (MC), and 25 (14.3%) had lymphocyte depletion (LD). The 8 patients with LP were all males; 49 (53.8%) of the patients with NS were males and 42 (46.2%) were females; 34 (66.6%) of the patients with MC were males and 17 (33.4%) were females; and 17 (68.0%) of the patients with LD were males and 8 (32.0%) were females. Of the 108 male patients, 8 (7.4%) had LP, 49 (45.3%) had NS, 34 (31.5%) had MC, and 17 (15.8%) had LD. Of the 67 females, 42 (62.7%) had NS, 17 (25.3%) had MC, and 8 (12.0%) had LD.

Previous treatment.—There were 3 distinct groups of patients: *a*) 45 patients with Stage III-B or IV disease who had received no previous treatment and in whom combination chemotherapy was the treatment of first choice; *b*) 62 patients who had received previous treatment with radiotherapy whose disease was either recurrent in a previously irradiated site or had extended beyond the generally accepted limits of radiation treatment, e.g., to the liver or lung; and *c*) 68 patients who had received previous treatment with single-agent chemotherapy with or without radiotherapy.

The distribution of patients by sex and previous treatment is shown in table 1. There was a similar proportional distribution of males and females in the 3 groups.

TABLE 1.—*Distribution of 175 patients by previous treatment and sex*

Group	Number of patients	Male (%)	Female (%)
No previous treatment	45	28 (62.2)	17 (37.8)
Previous radiotherapy	62	40 (64.5)	22 (35.5)
Previous chemotherapy ± radiotherapy	68	40 (58.8)	28 (41.2)
Total	175	108 (61.7)	67 (38.3)

Stage of disease.—The Rye system of staging was used (*6*). Previously untreated patients were staged immediately before chemotherapy was started. The stage to which previously treated patients were assigned was obtained by restaging immediately before the start of chemotherapy. Staging of patients was clinical and not pathologic, and their distribution by stage and sex is shown in table 2. There were 135 patients in Stages III-B or IV. All 40 patients in Stages II and III-A had been treated with previous radiotherapy, and their disease had recurred in a treated site.

TABLE 2.—*Distribution of patients by stage of disease and sex*

Stage	Number of patients		
	Total	Male	Female
I-A	0	0	0
I-B	0	0	0
II-A	7	5	2
II-B	6	2	4
III-A	27	16	11
III-B	42	29	13
IV-A	25	11	14
IV-B	68	45	23
	175	108	67

Investigations.—The following investigations were done before a patient received chemotherapy: 1) full clinical examination; 2) hemoglobin and total white blood cell count and differential, platelet count; 3) erythrocyte sedimentation rate; 4) aspiration bone-marrow biopsy; 5) determination of serum alkaline phosphatase, serum glutamic pyruvic transaminase, bilirubin, and total serum protein and electrophoreses; 6) determination of blood urea and uric acid; 7) posteroanterior chest X-ray and penetrated posteroanterior view; 8) bipedal lymphography; and 9) liver and spleen scans.

RESULTS

Remission Rates

Complete remission was defined as complete disappearance of all measurable evidence of disease, with resolution of all symptoms and laboratory evidence suggesting Hodgkin's disease.

The category "partial remission" is not used here, since we believe it to be synonymous with continuing disease activity.

The overall complete remission rate was 76.6%, in accord with our previous findings and those of DeVita et al. (*1*). Table 3 shows the remission rates for the 3 groups of patients. The highest complete remission rate of 87.1% was seen in the previously irradiated patients and may be due to a bias introduced by those patients with recurrent Stage II and III-A disease in whom a complete remission rate of 85% (34/40) was seen. Table 4 shows remission rate by stage of disease.

Histology at the time of treatment seemed to have little influence on remission rate, though there was a slight but not significant falling off of remission rate with worsening histology (table 5).

When the age of the patient and the presence or absence of classical symptoms of Hodgkin's disease symptoms were considered, they also had no significant influence on remission rate (tables 6, 7).

The remission rate for males was 83% and for females 65.7% in this series. This is curious in view of the greater frequency of NS among female patients, since this histologic type of disease is thought to be associated with a good prognosis, although this may only apply to patients in Stages I and II (7).

TABLE 3.—*Response to treatment*

Group	Complete remission (%)		Alive at time of analysis	Dead
No previous treatment	35/45	(77.8)	40/45	5
Previous radiotherapy only	54/62	(87.1)	51/62	11
Previous chemotherapy ± radiotherapy	45/68	(66.2)	43/68	25
Total	134/175	(76.6)	134/175	41/175

TABLE 4.—*Remission rates in the clinical stages of Hodgkin's disease*

Stage	Complete remission rate	Percent
I-A	—	—
I-B	—	—
II-A	7/7	100.0
II-B	4/6	66.6
III-A	23/27	85.2
III-B	36/42	85.7
IV-A	18/25	72.0
IV-B	46/68	67.6

TABLE 5.—*Remission rates in the histologic grades of Hodgkin's disease*

Histology	Patients	Complete remissions	Remission rate (%)
LP	8	7	87.5
NS	91	71	78.0
MC	51	37	72.5
LD	25	19	76.0
Total	175	134	76.6

TABLE 6.—*Influence of age on remission rate*

Age (yr)	Complete remission rate	Percent
<40	94/119	79.0
>40	40/56	71.4

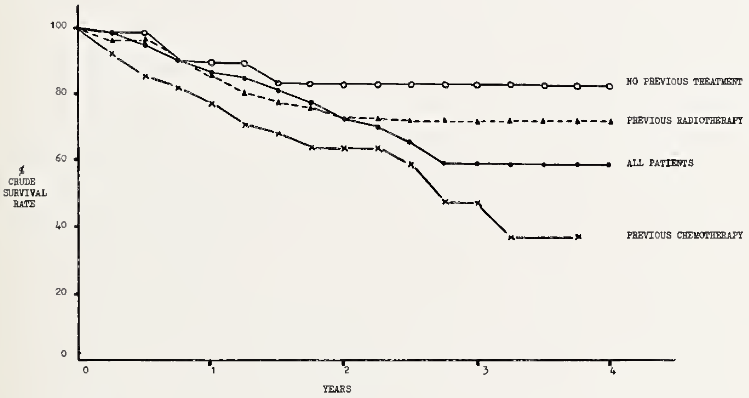
TABLE 7.—*Influence of symptoms on remission rate*

	Complete remission rate	Percent
A—No symptoms	48/59	81.3
B—Symptoms	86/116	74.1

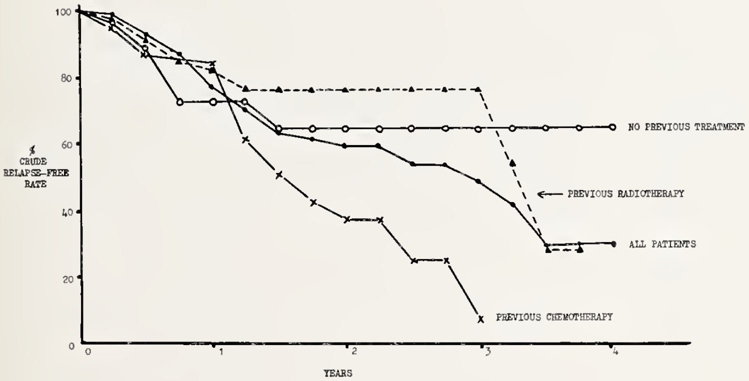
Survival

Survival has been calculated by the life-table method. The number of patients is so small after 3 years that estimates beyond this time are not reliable. Survival for all patients and for the 3 treatment groups is shown in text-figure 2.

Predicted survival for all patients at 3 years is 59.2%. For those patients who had received no previous treatment and for those who had received previous radiotherapy only, predicted 3-year survivals are 83.7 and 71.9%, respectively. Previous single-agent chemotherapy seems to prejudice survival with combination therapy, the predicted 3-year survival in this group being only 47.1%. This fact is even more striking if relapse-free survival is considered (text-fig. 3). The predicted 3-year relapse-free survival for the 134 patients who achieved complete remission is 48.2%. It is 64.2% for previously untreated patients and 76.6% for those previously irradiated, but it is only 7.9% for the group who received previous single-agent chemotherapy. Since the median survival of patients treated with single-agent chemotherapy has almost always been <24 months in the past (2) it now seems certain that previous single-agent chemotherapy is prejudicial to patient survival, even if this treatment is followed by combination therapy when relapse occurs.



TEXT-FIGURE 2.—Survival on MVPP related to previous treatment.

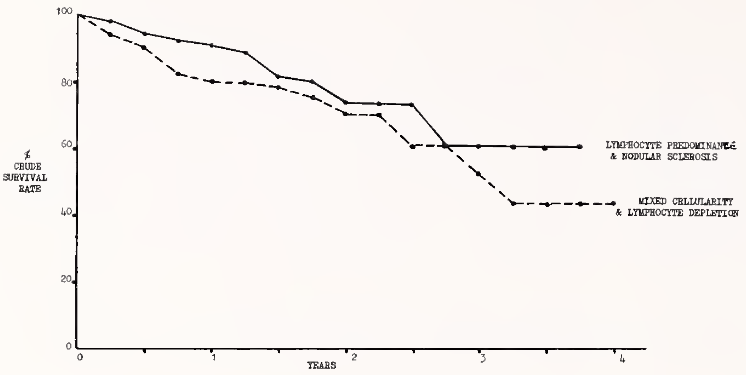


TEXT-FIGURE 3.—Relapse-free survival on MVPP related to previous treatment.

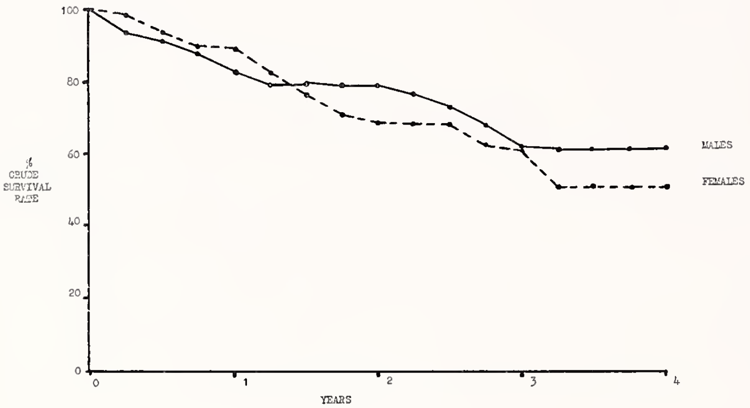
At 3 years, histology seems to have had little influence on patient survival (text-fig. 4), and sex has had no influence (text-fig. 5). However, there is a suggestion that the curve for the LP and NS group may be flattening out, whereas that for MC and LD is continuing to fall. If survival for all histologic types of Hodgkin's disease continues the same for patients on maintenance therapy, this may have important implications for the selection of future patients for chemotherapy.

Drug Dosage and Toxicity

In general, most patients have found the combination tolerable. Only one patient has refused treatment, due to a morbid fear of needles. Almost always transient, the nausea and vomiting produced by nitrogen mustard can be considerably relieved by the prior administration of chlorpromazine. Many patients have said that the second injection of mustine on day 8 pro-



TEXT-FIGURE 4.—Survival on MVPP related to histology.



TEXT-FIGURE 5.—Survival on MVPP related to sex.

duces far less nausea than the injection given on day 1, but we have no explanation for this.

Prednisone at the relatively low dose used in the combination has resulted in few side effects. Many patients have reported a transient feeling of depression and lack of energy when the drug is stopped abruptly on day 14, and some of them preferred reduction of the drug over a few days. Cushingoid signs and symptoms have been negligible. While taking prednisone, 4 patients complained of exacerbations of acne; in 2 patients, both diabetics, it was believed wisest not to give the drug at all.

Neurotoxicity was not a problem with vinblastine. Only one patient, a woman of 26 years, was severely incapacitated, developing foot drop and weakness of the arms after the fifth course of treatment. This condition cleared up and she has now fully recovered. In no other patient has neurotoxicity amounted to more than parathesiae and loss of tendon jerks which have tended to disappear in all patients after administration of the drug was stopped. No patient has been epilated. We consider that vinblastine is superior to vincristine in this combination because it is the neurologic side effects which are most distressing to the patients and every effort should be made to prevent their occurrence. The major toxic side effect of the combination is depression of the bone marrow. This depression tends to occur more frequently as the number of courses given increases and is more common in patients who have received previous single-agent chemotherapy or total-nodal irradiation. The nadir of the leukocyte and platelet counts tends to occur on about the 14th day from the start of treatment, and thus we have frequently been unable to give the third injection of vinblastine which is due at this time. The percentage of the calculated dose of mustine, procarbazine, and vinblastine given per course of treatment in the 3 treatment groups is shown in tables 8–10. Most patients received full doses of mustine and procarbazine in all

TABLE 8.—*Mustine—Percentage of calculated dose given per course of treatment*

	Course No.						
Group*	1	2	3	4	5	6	6 courses
NPT (45)	97.1	96.6	94.0	96.2	92.3	93.8	95.0
RT (62)	95.1	95.5	91.4	80.0	85.6	85.3	88.8
CT±RT (68)	95.6	84.3	90.5	86.3	74.1	91.4	86.9

*NPT: no previous treatment; RT: previous radiotherapy; and CT±RT: previous chemotherapy plus or minus previous radiotherapy.

TABLE 9.—*Procarbazine—Percentage of calculated dose given per course of treatment*

	Course No.						
Group*	1	2	3	4	5	6	6 courses
NPT (45)	97.0	96.3	94.7	96.8	99.0	94.4	96.3
RT (62)	99.6	93.4	96.0	97.8	97.2	93.7	96.3
CT±RT (68)	98.4	95.7	94.8	94.2	92.1	90.8	94.3

*For abbreviation *see* footnote table 8.

TABLE 10.—*Vinblastine—Percentage of calculated dose given per course of treatment*

	Course No.						
Group*	1	2	3	4	5	6	6 courses
NPT (45)	90.9	87.6	81.7	72.3	70.3	61.3	77.4
RT (62)	78.6	76.3	74.4	70.7	71.6	55.4	71.2
CT±RT (68)	74.9	69.5	65.5	65.2	58.5	60.8	65.7

*For abbreviation *see* footnote table 8.

TABLE 11.—*Proportion of vinblastine treatment dose not given due to neutropenia or thrombocytopenia*

	Day		
Group*	1	8	14
NPT (45)	14/196 (7.1%)	22/196 (11.2%)	108/196 (55.1%)
RT (62)	24/284 (8.4%)	29/284 (10.2%)	166/284 (58.8%)
CT±RT (68)	27/238 (11.3%)	33/238 (13.7%)	146/238 (61.3%)

*For abbreviation *see* footnote table 8.

6 courses, but only about two-thirds of the projected dose of vinblastine could be given. Table 11 shows that this was mainly due to treatments not given on day 14 because of depression of the blood count at that time. For this reason we have now abandoned the day-14 dose of vinblastine.

DISCUSSION

Undoubtedly, the MOPP combination is the most effective chemotherapeutic regimen available at present for the treatment of Hodgkin's disease. The MVPP combination appears to be comparable to it in terms of remission rate and survival in patients with advanced disease, the main difference between the combinations relating to their toxicity to the bone marrow and nervous system. We believe that the lack of neurotoxicity in the MVPP combination offers advantages in the management of previously untreated patients for whom combination chemotherapy is the treatment of first choice. In patients who have received previous radiotherapy, particularly so-called "total nodal," the relative bone-marrow sparing properties of MOPP would seem to offer a significant advantage over MVPP.

The problems of what to do after 6 courses of 4-drug chemotherapy remain unresolved. A current British Medical Research Council trial is comparing 4-drug maintenance given at 3-month intervals after 6 courses of MVPP, with 2-drug maintenance using vinblastine and procarbazine given in the same doses and at the same intervals as those 2 drugs in the 4-drug combination. This may indicate if less toxic maintenance is comparable with MVPP maintenance; however, whether any kind of maintenance therapy after 6 courses of treatment prolongs survival is still unknown.

Furthermore, maintenance does not appear to prolong disease-free survival in the previously untreated group of patients in whom the predicted relapse-free survival of 64.2% at 3 years is virtually the same as that produced in the patients receiving the original MOPP combination who were unmaintained after 6 cycles of treatment (*1*). Clearly, from these data, it is desirable to have at least some future trials of combination chemotherapy in which an unmaintained group is included if this question is finally to be resolved. Hersh et al. (*8*) have recently shown that 9 maintenance courses of MOPP given at 2-month intervals after complete remission had been achieved produced 73% disease-free survival at 35 months compared with 30% in patients who received no maintenance. These data seem to indicate that the timing of maintenance treatment is as important as the drugs in the combination.

Since the MOPP and MVPP combinations seem to be at present the most effective chemotherapy for advanced Hodgkin's disease, what to do in the future is a difficult question. Clearly, as new drugs become available they will need to be evaluated both alone and in combination. Studies on modification of drug doses and timing in the 2 original combinations may also prove fruitful.

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Clinical Trials With Intensive Chemotherapy and Radiotherapy in Hodgkin's Disease[1]

Mario De Lena, *M.D.*, Silvio Monfardini, *M.D.*, Gianni Beretta, *M.D.*, Franca Fossati-Bellani, *M.D.*, *and* Gianni Bonadonna, *M.D.*,[2,3] *National Cancer Institute, Milan, Italy*

SUMMARY—The preliminary results of an intensive treatment program for Stages II-B, III-B, III_S, and IV Hodgkin's disease are reported. The study was designed to explore the possibility of treating malignant lymphomas by a technique similar to that successfully used in treating acute leukemia. The protocol included an induction phase with multiple drug combination followed by a consolidation phase with high-energy irradiation (only for Stages II-B, III-B, III_S, and selected Stage IV patients). Maintenance therapy was carried out with sequential cyclic chemotherapy periodically interrupted by 1 cycle of reinduction. After observing the toxic and therapeutic results of a pilot study in which the induction was performed in 17 patients with quadruple chemotherapy (mechlorethamine, adriamycin, vincristine, and prednisone), we started a new trial in December 1970. The induction phase included 5 drugs: mechlorethamine, adriamycin, bleomycin, vincristine, and prednisone administered in 6 or 2 cycles. A total of 41 patients could be evaluated. Complete remission occurred in an average of 64% (68% after 6 cycles and 56% after 2 cycles). Complete remissions rose to 93% when partial plus complete responders were irradiated after induction chemotherapy. An adequate evaluation of the duration of complete remission is not yet available (median 6 months, as of March 1972). Both incidence and degree of toxicity were acceptable, and only a small percentage of the patients required a drastically reduced dosage. Furthermore, patients splenectomized showed less leukopenia compared to those not splenectomized before therapy. About 20% of the patients had slight pulmonary toxicity induced by bleomycin. In all patients, toxic lesions were reversible.—Natl Cancer Inst Monogr 36: 403–420, 1973.

INTENSIVE COMBINATION chemotherapy for advanced malignant lymphomas, in particular for Hodgkin's disease, has now overcome the obstacle of the initial clinical trials in which the primary goal was to produce a high percentage of complete remissions within a relatively short period and with minimal toxicity. But we are now at the stage where ideas, programs, and controlled studies are being focused on the effort to prolong the control of the disease in patients achieving an initial complete remission (*1*). In fact, the pioneer work of the group at the National Cancer Institute (*2, 3*) followed by other similar studies (*4–7*) has clearly demonstrated that the appropriate combination of some of the effective drugs available—a combination of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) and a combination of intravenous cyclophosphamide and vincristine (Oncovin) and oral prednisone (COP)—administered in full or nearly full dosage and through an intermittent dose schedule, produces complete remissions at an incidence and duration which appear significantly superior to those obtained in patients treated sequentially with single agents. Furthermore, by use of a dosage-attenuation schedule for myelosuppressive agents, treatment could be carried out continuously for 6 months with an acceptable toxicity (2).

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Presented by Dr. Bonadonna. *Address reprint requests to*: Dr. Gianni Bonadonna, Via Venezian, 1, Milan 20133, Italy.

[3] We are grateful to the many clinical associates and, in particular, to the radiation therapists who participated in the care of these patients. The cooperation of Mary Marchesi, R.N., has been invaluable. We also thank S. Ceccarini for preparing the graphs.

Survival after a 6-month course has been impressively long, especially in the initial series of untreated patients with Hodgkin's disease reported by DeVita and Carbone (*8*). In fact, 43% of the patients remained continuously free of the disease for periods up to 6 years from the last day of treatment. The median duration of complete responders was 36 months. In the more recent study by Luce et al. (*5*), Hodgkin's disease patients with little or no prior treatment had an estimated median survival time of 32 months.

The choice of drugs to be included in the combination varied slightly in the different studies. An alkylating agent, usually mechlorethamine (NH2) or cyclophosphamide, was always included because of its well-known effectiveness against tumors of the lymphoreticular tissue. Vincristine (VCR) was selected rather than vinblastine (VLB) in some trials (*2, 5*) because VCR had a moderate to mild myelosuppressive action; in other studies VLB was used instead (*4, 6, 7*). Corticosteroids were used in all studies because of their prompt effect with no myelosuppression. Procarbazine was the fourth agent almost always used, since a number of studies have established that it has a significant antitumor effect on Hodgkin's disease. There is no cross-resistance among the different drugs.

Since January 1970 we have been attempting to explore the possibility of treating malignant lymphomas by a technique similar to that successfully used in treating acute lymphoblastic leukemia of children (*9, 10*). Besides patients with Stage IV disease, patients with Stages II-B, III-B, and III_S diseases were selected, since in these patients radiotherapy alone is less effective in producing long-term survivors than in patients with the same stage but without systemic symptoms or splenic involvement (*11*). To see whether this method could be safely tolerated, we deliberately planned in a pilot study to give combination chemotherapy during the induction phase only for 4 weeks, to be followed by radiation therapy as consolidation treatment. Maintenance therapy was carried out with sequential cyclic chemotherapy, periodically interrupted by a course of inducer agents (reinduction). Among drugs effective in Hodgkin's disease were selected those—HN2, VCR, adriamycin (ADM), and prednisone—able to produce a prompt effect in responsive patients. ADM was included in the protocol because it produces in Hodgkin's disease a 75% I response (Karnofsky's scale) at a relatively low total dosage (*12, 13*). During the maintenance phase, cyclophosphamide, VLB, procarbazine, and methotrexate (MTX) were used in that order. They are all effective against Hodgkin's disease (*14, 17*), and there is no cross-resistance among these drugs (*15, 17*).

After observing that both radiotherapy and sequential chemotherapy were relatively well tolerated after a 4-week drug combination at reduced doses, we started a second trial in which treatment during the induction phase was intensified in terms of number of drugs, dosage, and period of time. Bleomycin (BLM) was included in the combination, since we found that it produced a 57% response rate for category I (*13, 18*) but no concomitant myelosuppressive effects. Induction was designed to be carried out through an intermittent drug treatment similar to that reported by DeVita et al. (2). Drugs were administered at a full-dose regimen, i.e., at a dose therapeutically effective when each agent is used alone.

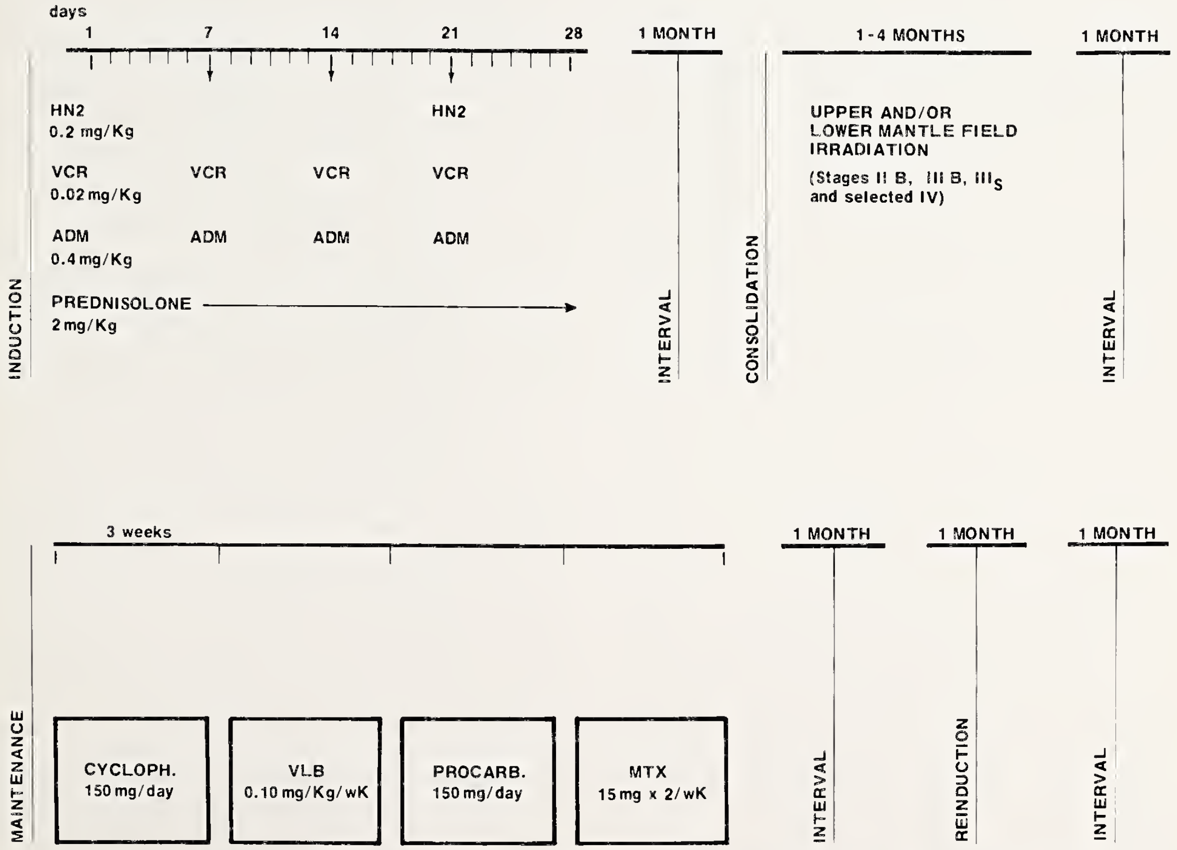
The present report aimed at the following: 1) to determine whether the 5-drug regimen with HN2, ADM, BLM, VCR, and prednisone (MABOP) could produce a response rate, in particular a complete remission rate, comparable to that produced by MOPP and COP; 2) to determine whether radiation therapy could increase the overall complete remission rate when complete plus partial responders with Stages II-B, III-B, and III_S were irradiated after the induction phase; 3) to compare in Stages II-B, III-B, and IV the incidence of complete remissions after 2 and 6 cycles of induction; and 4) to determine whether patients splenectomized before treatment could tolerate more chemotherapy and radiotherapy in comparison to those not splenectomized.

Before reporting the data on the 5-drug combination, we will briefly summarize the results obtained with the quadruple combination.

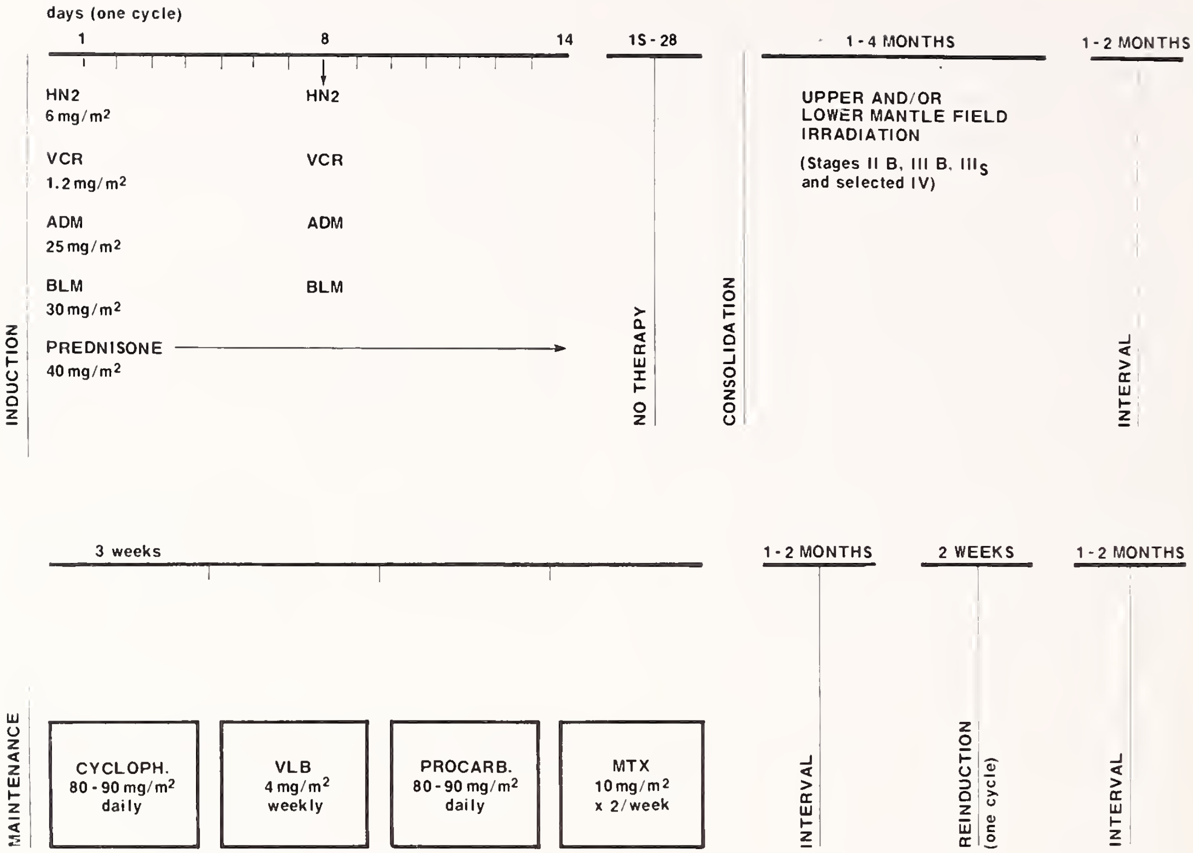
OUTLINE OF TREATMENT PROGRAM

Induction with 4-drug combination.—The protocol started in January 1970 is outlined in text-figure 1. The approach to the treatment program was deliberately cautious. The intention was to shrink the tumor considerably within a relatively short period without producing severe myelosuppression before starting radiation therapy. For this reason, with the exception of HN2, VCR and ADM were not administered at the maximum tolerated dose for a period of 1 month. But prednisone was given intramuscularly at a high dose which was reported to be effective by Hall et al. (*19*). After the induction phase, an interval of 1 month was instituted to allow recovery from the possible marrow toxicity and to assess the degree of remission. Radiation with ^{60}Co (3500–4000 rads) was delivered to all involved and to all proximal lymph-node-bearing areas. Then 4 weeks after irradiation, maintenance treatment was started with 4 drugs given in a sequential, cyclic fashion. Only VLB was administered intravenously; cyclophosphamide, procarbazine, and MTX were taken orally. Again, all drugs were not given at the maximum tolerated dose for a certain period of time. The maintenance phase was periodically interrupted by a cycle of reinduction for 4 weeks. The reinduction was carried out with the same drugs and the same dosage used during the induction phase.

Induction with 5-drug combination.—This


days
1
7
14
21
28
HN2
0.2 mg/Kg
HN2
VCR
0.02 mg/Kg
VCR
VCR
VCR
ADM
0.4 mg/Kg
ADM
ADM
ADM
PREDNISOLONE
2 mg/Kg
INDUCTION
1 MONTH
INTERVAL
CONSOLIDATION
1-4 MONTHS
UPPER AND/OR
LOWER MANTLE FIELD
IRRADIATION
(Stages II B, III B, III$_S$
and selected IV)
1 MONTH
INTERVAL
3 weeks
MAINTENANCE
CYCLOPH.
150 mg/day
VLB
0.10 mg/Kg/wK
PROCARB.
150 mg/day
MTX
15 mg x 2/wK
1 MONTH
INTERVAL
1 MONTH
REINDUCTION
1 MONTH
INTERVAL

TEXT-FIGURE 1.—Outline of the protocol employing a 4-drug combination during the induction phase.


days (one cycle)
1
8
14
HN2
6 mg/m2
HN2
VCR
1.2 mg/m2
VCR
ADM
25 mg/m2
ADM
BLM
30 mg/m2
BLM
PREDNISONE
40 mg/m2
INDUCTION
15-28
NO THERAPY
CONSOLIDATION
1-4 MONTHS
UPPER AND/OR
LOWER MANTLE FIELD
IRRADIATION
(Stages II B, III B, IIIS
and selected IV)
1-2 MONTHS
INTERVAL
3 weeks
MAINTENANCE
CYCLOPH.
80-90 mg/m2
daily
VLB
4 mg/m2
weekly
PROCARB.
80-90 mg/m2
daily
MTX
10 mg/m2
x 2/week
1-2 MONTHS
INTERVAL
2 WEEKS
REINDUCTION
(one cycle)
1-2 MONTHS
INTERVAL

TEXT-FIGURE 2.—Outline of the protocol employing a 5-drug combination during the induction phase.

study was started in December 1970. The different phases of treatment and the outline of the protocol are presented in text-figures 2 and 3. The experience gained with our 4-drug combination in terms of toxicity and remission rate suggested that patients should have been more intensively treated during the induction phase to achieve a higher percentage of complete remissions. Furthermore, other studies (*2, 5, 20*) clearly showed that patients could tolerate higher doses of some myelosuppressive agents without developing harmful toxicity when these agents were administered intermittently in cyclic combination. The 5-drug combination reported in text-figure 2 is the most recent version of the protocol which is currently being employed in our Institute. In fact, at the beginning of the study, to simplify drug administration, this sequence was almost immediately abandoned. The induction phase was carried out with either 6 or 2 cycles. In patients of the first group, BLM and corticosteroids were given only in cycles 1, 3, and 5. In patients of the second group, they were administered in 2 cycles. The sequence, intensive chemotherapy (induction)—radiotherapy (consolidation)—sequential cycling maintenance—reinduction (1 cycle), is the same as that outlined in the previous protocol. However, during the induction phase, 2 weeks of therapy (day 1–14) were alternated with 2 weeks of no therapy (day 15–28) in each cycle, as carried out by DeVita et al. (*2*). Furthermore, a rest period of 2 months preceded radiotherapy. Also an interval of 1–2 months preceded and followed maintenance therapy and reinduction.

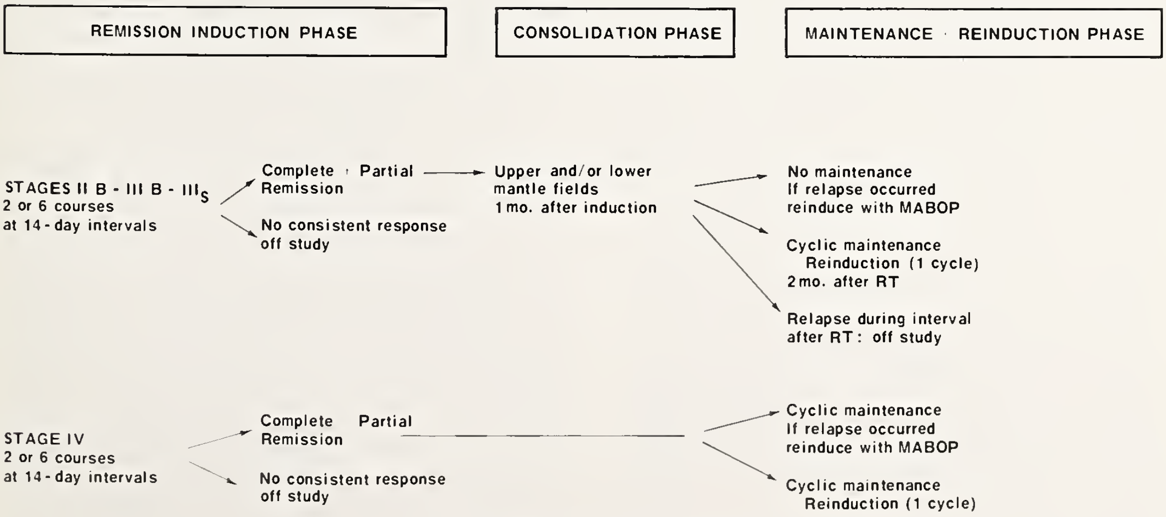
Text-figure 3 outlines the entire study. Patients suitable for intensive therapy were those with Stages II-B, III-B, III$_S$ and IV (A and B). Furthermore, all patients with histologic evidence of vascular invasion should be included in the protocol even though they have no systemic symptoms. Unless unequivocal signs of visceral or distant bone involvement are present, patients should be subjected to diagnostic laparotomy with open iliac crest bone-marrow biopsy. For the induction phase, patients were allocated to 6 or 2 cycles of 5-drug combination. The remission induction treatment required repeated blood counts and constant control by experienced physicians. Thus the randomization was not blind, but to the 6-cycle induction therapy were assigned those patients who could come at least twice a month to the out-patient clinic to receive the drug administration. In contrast, the 2-cycle induction was carried out on patients living too far from the Institute to regularly attend the clinic for a prolonged period. In our opinion, the induction phase of this treatment cannot be carried out by local physicians. Since the National Cancer Institute is a referral center, about 50% of the patients admitted with the clinical diagnosis of malignant lymphoma come from different areas of the country. In contrast, at least 30% of the patients actually living in Milan or in its suburbs have immigrated from other provinces. Although admittedly this does not represent the ideal type of randomization, this study can be carried out with a much larger population.

Patients with Stages II-B, III-B, and III_S achieving a complete or partial remission were subsequently irradiated as described. Those who did not show a consistent response after induction (regression 50%) were removed from the study. After irradiation, patients were randomly allocated to either no further treatment or to continued treatment. Patients were stratified according to the 3 stages and to the 4 histologic types, thus forming 12 subgroups. Within each subgroup, each successive pair of patients received either no maintenance therapy or maintenance treatment plus reinduction. The type of treatment assigned to each member of the pair was randomly chosen.

Patients with Stage IV disease were allocated to 6 or 2 cycles according to the criteria of geographic distribution as described. Those achieving complete and partial remission were randomly allocated to either cyclic maintenance only or to maintenance plus reinduction. Stratification takes into account the number of extranodal involvements (one involvement vs. more than one involvements). Subgroups were also formed to assign to maintenance therapy or to maintenance plus reinduction the different dominant organ lesions (e.g., liver, lung, bone, skin) when they appeared to be unique.

PATIENTS AND METHODS

From January 1970 to February 1972, 82 unselected consecutive patients were entered into


REMISSION INDUCTION PHASE
CONSOLIDATION PHASE
MAINTENANCE REINDUCTION PHASE
STAGES II B - III B - III S
2 or 6 courses
at 14-day intervals
Complete + Partial Remission
No consistent response off study
Upper and/or lower mantle fields
1 mo. after induction
No maintenance
If relapse occurred reinduce with MABOP
Cyclic maintenance
Reinduction (1 cycle)
2 mo. after RT
Relapse during interval after RT: off study
STAGE IV
2 or 6 courses
at 14-day intervals
Complete Partial Remission
No consistent response off study
Cyclic maintenance
If relapse occurred reinduce with MABOP
Cyclic maintenance
Reinduction (1 cycle)

TEXT-FIGURE 3.—Outline of study.

the study (17 for induction with 4-drug combination and 65 for induction with 5-drug combination). Only 64 patients could be considered in the present report; the remaining 18 patients are still receiving the induction treatment. Patients were admitted to the hospital for initial work-up. Induction and consolidation phases were carried out in the hospital only on patients living far from the Institute. Otherwise, treatment was administered on an ambulatory basis.

Patient sample.—Most patients were referred from different regions of the country. Those who had received prior radiotherapy above and below the diaphragm were excluded from this study, as were those heavily treated with several chemotherapeutic agents and known to have become resistant to them. But patients treated with limited radiotherapy (usually with nonradical doses), those with disease relapse into an organ system after upper or lower mantle field irradiation, or those who had received inadequate courses of single drugs were entered into the study. Of the 17 patients accepted for the study of induction with 4-drug therapy, 1 was pretreated with radiotherapy and 10 with chemotherapy plus limited irradiation. Their average age was 28 years (6–53 yr); 10 were males and 7 females. A total of 47 patients (26 males and 21 females) were entered into the study for the evaluation of the 5-drug combination. Their average age was 39.5 years (10–62 yr). A total of 22 were untreated, 11 had received radiotherapy, and 8 had chemotherapy plus small doses of radiation. All patients had adequate bone marrow reserve, as defined by white blood cell counts of 4500/mm^3 or greater, platelet counts of $\geqq$150,000/mm^3, and normocellular marrow (aspiration and/or biopsy). In 20 patients the granulocyte reserve was tested with ethiocolanolone (0.1 mg/kg intramuscularly) before therapy and periodically after the different phases of treatment. Table 1 reports the dosage-attenuation schedule adopted in the presence of myelosuppression.

TABLE 1.—*Dosage-attenuation schedule for bone marrow depression*

Nadir leukocytes	Nadir platelets	Dosage adjustment
>4000/mm^3	>100,000/mm^3	100% of all drugs
3999–3000/mm^3		100% VCR, BLM, prednisone 50% HN2, ADM
2999–2000/mm^3	100,000–50,000/mm^3	100% BLM, prednisone 50% VCR 25% HN2, ADM
1999–1500/mm^3		100% BLM, prednisone 25% VCR No HN2 and ADM
<1500/mm^3	<50,000/mm^3	100% BLM, prednisone No HN2, ADM, and VCR

Histologic diagnosis.—All patients had histologically proved diagnoses of Hodgkin's disease. All slides of lymph node biopsies (carried out elsewhere) were reviewed by pathologists of the Institute. The morphologic classification used for all tissues removed during the initial diagnostic workup was that proposed at Rye (*21*), with the recommendations added at Ann Arbor (*22*).

Staging.—The evaluation procedures used were those recommended at Ann Arbor (*23*). In particular, diagnostic laparotomy with splenectomy was performed in 3 of 17 patients of the first group (induction with quadruple therapy) and in 23 of 41 patients of the second group (induction with 5-drug combination). Drill bone-marrow biopsy was performed only in the last 9 patients; isotopic scanning of liver and spleen with ^{198}Au was done in a total of 52 of 58 patients. Almost all patients not subjected to diagnostic laparotomy had liver biopsy either with the use of peritoneoscopy or a Menghini needle. Strontium-85 bone scans were performed only if skeletal survey was not conclusive for metastatic bone involvement. Lymphangiograms were always reviewed by a radiologist. The delayed hypersensitivity (to purified protein derivative and dinitrochlorobenzene) was tested in 36 of 58 patients. After the diagnostic studies, patients were classified as to either clinical stage (no laparotomy) or pathologic stage (laparotomy) as proposed by the Committee on Hodgkin's Disease Staging Classification (*24*).

Assessment of results.—Periodically, during the whole period of induction, physical examination, radiologic studies, and laboratory tests were performed to determine the degree of tumor regression. In particular, during the interval after induction, consolidation, maintenance, and reinduction, a thorough re-evaluation was carried out to document the clinical absence or presence of Hodgkin's disease. Remissions were characterized as complete or partial. A complete remission was defined as the absence of any clinical evidence of disease (apparent total regression of all lesions and disappearance of all symptoms). We found it difficult to evaluate the response to chemotherapy by following serial flat plates of the abdomen in patients who had a lymphangiogram. In fact, if disease had completely disappeared elsewhere and the response seemed to be prompt and adequate, sometimes one could observe an incomplete shrinkage of retroperitoneal adenopathy. Since this can be noticed even after radical doses of irradiation (*25*), we believe that perhaps the lymph node is permanently distorted by the lymphoma and/or by the dye and that the disease may well have been eliminated in the sites. Thus we have also called these patients complete responders, though obviously proof would lie in laparotomy and biopsy. A partial remission was defined by a decrease of 50% in the product of 2 diameters of all measurable lesions, lasting at least 1 month after the end of induction or of consolidation. Tumor regression <50% by the end of induction was categorized as failure.

For the present report, all 17 patients entered into the study with a quadruple drug combination could be evaluated. Only 41 of 47 of those who belonged to the group given the 5-drug combination could be evaluated. In fact, in 2 patients, no clear-cut objective parameters of drug response could be found from retrospective analysis; 2 patients refused further treatment; 1 patient died of sepsis within 1 week after starting therapy. Of the 41 patients who completed the induction phase, 15 have also completed the irradiation program as of February 1, 1972. Only 6 patients are presently in the maintenance phase, and 2 have been subjected to reinduction.

RESULTS

4-Drug Combination

The response to induction is presented in table 2. A total of 8 of 17 patients (47%) achieved complete remission. Interestingly, 6 of 8 patients had received prior therapy and 3 of 8 had Hodgkin's disease, lymphocytic depletion type. In all responsive patients, the lesions promptly shrunk, and tumor regression usually was the greatest after the 4th week of therapy. A minimal evidence of recurrent tumor was observed in 2 patients during the 4-week interval before radiotherapy. A total of 13 patients (7/8 having achieved complete remission and 6/7 having achieved partial remission) were consolidated with radiotherapy. After irradiation, 11 of 13 patients (85%) were in complete remission, 1 was in partial remission, and 1 had a mixed response. Complete and partial responders (12 patients) were then started on cyclic maintenance plus reinduction. Table 3 summarizes the therapeutic results obtained with this combined treatment. One patient had a relapse during radiotherapy (mixed response) and 2 others during maintenance treatment. As of March 1972, 53% of the patients are still in complete remission, with a median duration of 14 months.

Toxicity during induction was moderate (leukopenia and paresthesias 24%, alopecia 18%, and diabetes and moon facies 6%). In 9 of 17 patients (53%), no consistent toxic signs were observed, with the exception of nausea and vomiting, especially after administration of HN2. Table 4 shows the incidence and the degree of myelosuppression during the different phases of treat-

TABLE 2.—*Response to induction (4-drug regimen)*

Stage	Patients	Response Complete remission	Partial remission	Remission-induction failures
II-B	2	2		
III-B*	9	3	5	1
IV	6	3	2	1
Total	17	8†	7	2

*1 III_E, 1 III_S.
†6/8 had prior chemotherapy or radiation therapy.

TABLE 3.—*Response to quadruple chemotherapy plus radiation therapy followed by cyclic maintenance and reinduction treatment (data as of March 1, 1972)*

Total patients treated	17	
Remission-induction failures	2	(12%)
Partial remissions	7	(41%)
Complete remissions after chemotherapy	8	(47%)
Complete remissions after chemotherapy + radiation therapy	11/13	(85%)
Median duration of complete remission	14	months
Relapse during radiotherapy	1	
Relapse during maintenance	2	
Median time to relapse	12.8	months
Total continuously free of disease*	9/17	(53%)
Total alive†	15/17	(89%)

*2 II-B, 6 III-B, 1 IV.
†Dead patients had lymphocyte-depleted Stage IV-B.

ment. While no consistent thrombocytopenia was detected, leukopenia was progressive from induction to consolidation as well as from the first to the third course of maintenance chemotherapy. But it occurred at a rather low incidence during reinduction, thus confirming that the dose schedule used was mild.

5-Drug Combination

The response to remission-induction treatment is presented in table 5. In this study, 68% of the patients given 6 cycles achieved complete remission as compared to 56% of those treated with only 2 cycles. The difference in the remission rate appeared considerable at a level of Stage IV (56% vs. 25%), though the number of patients was too small to draw conclusions. Tables 6 and 7 report the response to treatment in relation to histologic classification and to prior therapy, irrespective of stage and number of cycles administered. Complete responders were distributed equally among all histologic groups, including the lymphocytic depletion type. But the lowest percent of complete responders (50%) occurred in the group of patients pretreated primarily with ·chemotherapy. Table 8 details the response to treatment and the duration of remissions after induction therapy (41 patients) and after induction plus consolidation (15 patients). A total of 26 of 41 patients (64%) achieved complete remission after either 6 or 2 cycles of combination chemotherapy. After subsequent irradiation, given so far

TABLE 4.—*Myelosuppression during the different phases of treatment*

Phases of treatment	Patients at risk	Number with myelo-suppression* (%)	Mean nadir White blood cells	Mean nadir Platelets
Induction	14	4 (24)	2800	180,000
Consolidation	13	6 (46)	2700	190,000
Maintenance after radiation therapy				
1st course	12	3 (25)	2200	170,000
2d course	12	5 (42)	2200	140,000
3d course	4	3 (75)	2700	150,000
Reinduction				
1st cycle	12	2 (17)	2700	160,000
2d cycle	9	1 (11)	2800	170,000

*Leukocytes <3000 and/or platelets <100,000.

only to a limited number of patients, the complete responders increased to 93% and only 1 of 5 partial responders failed to show complete regression after radiotherapy. Although only 6 patients (4 with Stage IV) have been started on maintenance therapy, none has shown recurrence or appearance of new manifestations. All patients are still alive as of March 1972, and 66% of them continue to be free of disease with a median duration of 6 months.

The types of toxic manifestation found during the induction phase are reported in table 9. When analyzed separately, the group given ADM for the first 3 days with BLM on day 4, 7, 10, and 13 and the group given all drugs but prednisone on day 1 and 8 failed to show significant difference with regard to acute and chronic toxicity. Therefore, toxic effects as therapeutic results are considered together. The most common side effect was the loss of hair almost essentially due to the administration of ADM, followed by the occurrence of skin hyperpigmented stria secondary to BLM. Bone marrow depression occurred in about half the patients treated with 6 cycles and in about one-third of those treated with 2 cycles. BLM-induced interstitial pulmonary lesions occurred in about the same percentage of patients despite the fact that, in patients treated with the 5-drug combination for a limited period of time, BLM was administered during both cycles. In all patients, lung lesions were very mild. In 9 of 10 patients, the reticulomicronodular pattern typical of early pulmonary involvement (*18*) was detected on repeated chest X-ray, and in the tenth patient only crepitant rales were present at both lung bases. All patients remained asymptomatic. Paresthesias caused by VCR were moderate in most patients. A much higher percentage of the patients lost deep tendon reflexes. Constipation and motor weakness were occasional but mild. Stomatitis, probably a secondary response to ADM and BLM, was also moderate. Amenorrhea was seen in 3 patients treated with 6 cycles, but it always occurred after the second cycle. Only 1 patient developed a peptic ulcer which became symptomatic about 2 months after induction; this patient required gastrectomy. All patients complained of considerable nausea and vomiting, which usually worsened despite adequate administration of sedatives with each exposure. In 3 patients, HN2 had to be replaced with equivalent doses of Thio-TEPA (12 mg/m^2).

Although, in general, treatment was well tolerated and all types of toxicity were reversible, bone marrow suppression and interstitial lung lesions represented the dose-limiting factors. Table 10 reports the dose reduction required for each drug in each cycle in relation to the projected full dosage. An average of 94% was administered during the whole period of induction carried out with 6 cycles. If only myelosuppressive agents (alkylating agent, ADM, and VCR) are considered, the average was 92%. This

TABLE 5.—*Response to induction (5-drug regimen)*

6 cycles					2 cycles				
		Response					Response		
Stages	Number of patients	Complete remission (%)	Partial remission	Remission-induction failures	Stages	Number of patients	Complete remission (%)	Partial remission	Remission-induction failures
II-B*	5	4 (80)	1		II-B†	5	2 (40)	3	
III-B‡	11	8 (73)	2	1	III-B§	7	6 (86)	1	
IV	9	5 (56)	2	2	IV	4	1 (25)	3	
Total	25	17 (68)	5	3	Total	16	9 (56)	7	

*1 II_E.
†2 II_E.
‡7 III_S.
§1 III_E and 2 III_S.

TABLE 6.—*Response to induction (6 and 2 cycles) in relation to histologic expression*

Histology	Number of patients	Response		
		Complete remission (%)	Partial remission	Remission-induction failures
Lymphocyte predominance	5	3 (60)	1	1
Nodular sclerosis	8	5 (63)	3	
Mixed cellularity	15	9 (60)	5	1
Lymphocyte depletion	13	9 (70)	3	1
Total	41	26 (64)	12	3

TABLE 7.—*Response to 5-drug combination in relation to previous therapy*

Type of therapy	Number of patients	Response		
		Complete remission (%)	Partial remission	Remission-induction failures
None	22	14 (64)	7	1
Radiotherapy	11	8 (73)	2	1
Chemotherapy (+ radiotherapy)	8	4 (50)	3	1
Total	41	26 (64)	12	3

TABLE 8.—*Response to 5-drug chemotherapy plus radiation therapy (data as of March 1, 1972)*

Total patients able to be evaluated	41	
Remission-induction failures	3	(7%)
Partial remissions	12	(29%)
Complete remissions after chemotherapy	26	(64%)
Complete remissions after chemotherapy + radiation therapy	14/15	(93%)
Median duration of complete remission	6	months
Relapse during radiotherapy	0	
Relapse during maintenance	0	
Total continuously free of disease	27/41	(66%)
Total alive	41/41	(100%)

TABLE 9.—*Toxic manifestations after 5-drug combination (induction phase)*

Type of toxicity	6 cycles (25 patients)		2 cycles (16 patients)	
	Number of patients	Percent	Number of patients	Percent
Alopecia	25	100	16	100
Skin changes	16	64	6	44
Myelosuppression	12	48	5	31
Paresthesias	7	28	2	13
Stomatitis	6	24	2	13
Interstitial pneumonia	5	20	4	25
Amenorrhea	3	12		
Peptic ulcer	1	4		

Table 10.—*Average of projected dose administered per cycle*

Drugs	Cycle						Average total course
	1	2	3	4	5	6	
	←			%			→
Prednisone*	100	—	100	—	100	—	100
BLM*	100	—	93	—	93	—	95
ADM	100	98	96	100	95	97	97
VCR	100	95	95	89	91	94	94
Alkylating agent	93	90	84	79	80	84	86
Total average	98	94	91	89	93	88	94

*On cycles 1, 3, and 5.

Table 11.—*Degree of myelosuppression during 5-drug combination*

Cycle	Mean nadir (white blood cells/mm³)	Patients with white blood cell counts (%)				Mean nadir platelets × 10³	Patients with platelet counts (%)	
		<1000	<2000	<3000	<4000		<50,000	<100,000
1	4000	0	17	19	7	205	0	0
2	4000	3	11	22	8	108	3	0
3	3900	0	9	35	9	165	0	0
4	3600	0	11	17	22	178	5	0
5	4100	0	7	28	0	198	0	0
6	4400	0	0	17	8	180	0	0

was higher than that reported by DeVita et al. (*2*) where 83% of the projected dose of the alkylating agent was actually given and then 92% of VCR (injected at a dose of 1.4 mg/m² rather than 1.2 mg/m²). During this initial part of the study, 14% of the patients did not require a dose reduction of myelosuppressive drugs. Drug reduction was required in 57% of the untreated patients and in 43% of the pretreated patients. The degree of myelosuppression observed during the induction is illustrated in table 11. Severe infections secondary to agranulocytosis occurred only in 1 patient (not included in the therapeutic evaluation because she died within 1 week from starting therapy). Since the patient was nephrectomized 4 weeks before, possibly inadequate drug excretion led to accumulation and increased early toxicity.

The incidence and the degree of leukopenia appeared definitely less pronounced irrespective of prior therapy in patients treated with 6 cycles and previously splenectomized in comparison to those with no splenectomy. This difference was not seen in patients given only 2 cycles of intensive chemotherapy, and there was no difference in the nadir of white blood cells even after consolidation with radiotherapy (table 12).

DISCUSSION

Combination chemotherapy and combination chemotherapy plus irradiation appear to represent a major area of contemporary clinical investigation for a number of tumors. The first experimental models for intensive drug treatment were provided about 10 years ago by Skipper et al. (*26*). The translation of his principles on cell kill and curability to the therapy

TABLE 12.—*Incidence and degree of leukopenia during induction (6 and 2 cycles) and consolidation in patients splenectomized and in those not splenectomized*

Treatment			6 cycles				2 cycles	
		Patients at risk	Mean nadir (white blood cells/mm^3)	Patients with white blood cells <3000 (%)		Patients at risk	Mean nadir (white blood cells/mm^3)	Patients with white blood cells <3000 (%)
Chemotherapy	Splenectomy	16	3700	53	Splenectomy	7	3500	33
	No splenectomy	9	1900	90	No splenectomy	9	3600	33
Radiotherapy	Splenectomy	7	3900	29	Splenectomy	6	2700	50
	No splenectomy	0	—	—	No splenectomy	2	3200	50

of human neoplasms was started with acute leukemia, and only during the past few years have different types of solid tumors been treated with multiple drug combinations (*27–29*).

Among the group of malignant lymphomas, Hodgkin's disease was the first to undergo experimental trials with single or multiple drug combinations preceded and/or followed by radiotherapy. The advances in the staging classification, the impressive results achieved with high-energy, intensive irradiation in Stages I, II-A, and III-A, and the introduction of new effective drugs stimulated medical oncologists to attempt a long-standing control of the disease also in its more advanced phases. The first pilot study was reported by Frei et al. (*30, 31*) who administered in 14 patients with Stages I, II, and III (A and B) three 14-day courses of cyclophosphamide, VCR, MTX, and prednisone. Radiotherapy (3600–4000 rads) was given between the first and the second cycle of chemotherapy. The overall complete remission induction was 86%. The mean duration of unmaintained remissions for Stages I and II disease was 32 months, and that for the Stage III group was 16 months. The combined treatment was well tolerated, and further intensive chemotherapy was possible after high-dose but limited field irradiation. This pilot study provided the clinical basis for the subsequent trials carried out by the group at the National Cancer Institute (*2, 3, 20*) and by the Southwest Cancer Chemotherapy Study Group.

The most recent experience is that gained by European investigators. Tubiana et al. (*32*) treated 70 patients with Stage I and II Hodgkin's disease with upper and/or lower mantle field irradiation (4000 rads). From 4 to 6 weeks after radiotherapy, patients in complete remission were randomized to no further treatment and to single drug therapy for 2 years (VLB, 10 mg/wk). Prolonged administration of chemotherapy decreased the incidence of relapse in nonirradiated and in irradiated areas, but only in patients with mixed-cellularity and lymphocytic depletion types; in patients with lymphocytic predominance and nodular sclerosis, no significant improvement was achieved. A similar study was performed on 150 patients by Teillet et al. (*33*). Patients with Stage I and II disease were given HN2 (0.5 mg/kg) followed by radiotherapy (4000 rads) and then by VLB administration (0.2 mg/kg/month) for 3 years. Although treatment was well tolerated, survival was not superior to that obtained by radiotherapy alone. However, Hancock and Ledlie (*34*) came to opposite conclusions.

On Stage III-B and selected Stage IV patients, a more aggressive approach was attempted by Amiel et al. (*6*). The protocol was designed so that patients would benefit from both chemotherapy and radiotherapy. In all 56 patients, treatment started with induction chemotherapy (VLB alone, 7.5 mg/m^2/wk, or procarbazine alone, 150 mg/m^2/day, or combined with VLB, triethylene melamine, and corticosteroids) and was followed by complementary radiotherapy (3500–4000 rads). After irradiation, 20 patients received supplementary chemotherapy (VLB, 5 mg/m^2 every 1–2 wk), and 8 patients were splenectomized. Complete remissions occurred in 12.5%, but 11 of 56 patients had severe leukopenia after the induction phase and had to be isolated in the pathogen-free rooms. After radiotherapy, complete remissions were 82% and did not appear to be markedly influenced by prior therapy. The median remission for Stage III patients was 28.5 months, and 15% still remain free of disease after 4 years. However, Stage IV patients all had relapses after 42 months. Jacquillat et al. (*7*) treated Stages III and IV with a 5-drug regimen including thio-TEPA (10 mg/wk), VLB (10 mg/wk), rufochromomycin (200 mg/wk), procarbazine (50–100 mg/day), and prednisone (1 mg/kg/day for 6 wk). Induction was continued for 3 months but was not followed by radiotherapy. Maintenance treatment was carried out with 6-mercaptopurine (45 mg/m^2/day) plus MTX (15 mg/m^2/wk) subsequently replaced by chlorambucil and procarbazine. Maintenance was periodically (2d, 4th, 7th, 12th, and 18th month) interrupted by a 2-week course of reinduction. The survival after 1 year was 86%. No other details were given.

The results of all these studies show that chemotherapy and especially combination chemotherapy, if properly combined with radiotherapy, effectively control local and advanced Hodgkin's disease. The sequence, chemotherapy—irradiation—chemotherapy, can increase the duration of unmaintained remissions, and if both dose schedule and rest periods are properly timed, a significant improvement can be achieved without severe marrow depression. Chemotherapy and radiotherapy given almost at the same time proved also to be useful (*35*), but their additive marrow toxicity limits a prolonged administration of high doses of drugs.

Our study was designed to produce maximum regression (induction) before extensive irradiation (consolidation) which was to be followed by further chemotherapy (maintenance + reinduction). Patients with Stage II-B and III-B Hodgkin's disease were selected because of inadequate long-term control by radiotherapy alone (*11*). Patients with Stage III_S disease were included, since splenic involvement may reflect potential concomitant liver involvement (*36*), as well as all localized cases with histologic evidence of vascular invasion. Also Stage IV patients with limited osseous, pulmonary, and cutaneous involvement may be suitable for irradiation after chemotherapy.

The rationale for giving combination chemotherapy before radiotherapy is as follows: 1) Chemotherapy can control disseminated foci clinically not detectable, such as in Stages II-B, III-B, and III_S; 2) bone marrow reserve recovers more quickly after intensive cyclic chemotherapy than after intensive radiotherapy (*37–39*); and 3) a partial remission after chemotherapy can become more easily complete after subsequent radiotherapy, rather than vice versa. That further chemotherapy should be administered after consolidation is supported by the experience achieved in other studies of acute leukemia (*9, 10*) and lymphomas (*5*). These studies showed that the duration of initial remission and of effective survival can be prolonged if maintenance therapy and reinduction therapy are instituted. In Hodgkin's disease with occult foci not included within the radiation field, maintenance plus reinduction chemotherapy would prolong the control of the disease and possibly in some cases eradicate the disease. Thus, during maintenance therapy, each drug was administered at a dose effective when used alone for a minimum of 3 weeks. Drugs are given in a sequential cyclic fashion, since, in patients with apparently complete remission, there are by definition no clinical parameters to judge drug response or resistance. The same applies for reinduction treatment with complete remission.

The results of our pilot study confirm that a higher percentage of complete remissions can be achieved when complete plus partial responders are irradiated after induction chemotherapy. These results question the opinion of Johnson and Brace (*40*) who reported a reduced radiation response after prior chemotherapy. Furthermore, radiotherapy preceded by induction chemotherapy and followed by repeated courses of sequential cycling treatment proved to be well tolerated, despite increased marrow toxicity after each course of maintenance therapy. It should be recalled, however, that the induction phase was performed with ADM and VCR at about 50% of the tolerated dose.

The 5-drug induction combination included 2 relatively new agents effective in Hodgkin's disease, ADM and BLM. As stated in the introduction, these drugs were selected in addition to HN2, VCR, and prednisone because they rapidly affect responsive patients, the average time to detect an appreciable response being about 2 weeks (*12, 13, 18*). BLM is also not myelosuppressive. All drugs included in our induction phase are independently active against Hodgkin's disease with no cross-resistance among them (*41*) and can exert a prompt effect in terms of days.

The results of combining 5 agents with peculiar inducer properties might explain why the maximum regression was observed almost always after the second cycle. We believe it is still too early to judge the actual contribution provided by ADM and BLM in producing complete remissions. However, irrespective of stage, histologic type, prior treatment, and administration of 6 rather than 2 cycles, our initial complete remission rate (64%) lies somewhat between that obtained by the MOPP of DeVita et al. (81%) and the COP of Luce et al. (36%). COP consisted of an alkylating agent (cyclophosphamide), VCR, and prednisone given for 6 cycles during the induction phase (*5*). Complete responses produced by COP were slightly more than half those obtained by our 5-drug combination which included ADM and BLM besides an alkylating agent (HN2), VCR, and prednisone. Obviously, this does not imply that a remission rate higher than that achieved by COP could not be obtained by adding to the combination agents other than ADM and BLM, e.g., procarbazine or BCNU.

Also, in our experience, the percentage of complete responders was slightly less in patients given prior chemotherapy (*4, 20*). On the contrary, as noted by DeVita et al. (*2*), all histologic types showed an almost equal percentage of response. The duration of unmaintained remission and the effect of maintenance plus reinduction on survival cannot yet be evaluated, since sufficient time has not elapsed to evaluate an adequate number of cases.

The incidence and degree of toxicity during the first 2 phases of treatment (induction and consolidation) was acceptable, and only a small percentage of patients required a drastically reduced dose. Even in those patients with pulmonary toxicity, interstitial lesions were discovered at early phases, remained asymptomatic, and always were reversible. Furthermore, our experience indicates that myelosuppression after combination chemotherapy followed by radiotherapy might be much less pronounced in patients in whom the spleen was removed during diagnostic laparotomy in comparison to that observed in nonsplenectomized patients. These data agree with those reported by other authors (*42, 43*) and favor the indications for splenectomy also to some good-risk patients with Stage IV disease.

The second part of the study intends first of all to compare, in Stages II-B, III-B, and III$_S$, the duration of remission produced by combination chemotherapy plus radiotherapy in patients maintained on sequential chemotherapy plus reinduction with those not maintained on treatment. In Stage IV will be compared the duration of remission in patients maintained on cyclic chemotherapy plus reinduction with the duration of remission in those maintained without reinduction. After the MOPP treatment, relapses were observed with a median duration of 11 months (*8*), but patients were not irradiated after intensive chemotherapy. Therefore, it is important to determine in both groups of patients treated with 2 or 6 cycles whether the relapse-free interval after chemotherapy plus radiotherapy is equal to or less than that obtained after more aggressive treatment prolonged for at least 2 years.

The survival of patients with Stages II-B, III-B, and III$_S$ will be further studied in relation to the 4 histologic types. It is well known that the different histologic types carry a different prognosis in Hodgkin's disease (*44, 45*). We believe it is essential to know if survival after chemotherapy plus irradiation is also related to histology. Finally, in Stage IV patients, the survival in relation to the presence of a single versus multiple organ involvement will be also compared. It is generally accepted that the more diffuse the lymphoma the lower the survival. However, it has not yet been determined whether the various types of Stage IV patients have a different prognosis in relation to different types of therapy, i.e., whether patients with different organ involvement (single or multiple) or patients with a different type of organ involvement (e.g., lung versus liver) respond differently to combination chemotherapy.

Thus, making the results of various types of studies (*46*) more adequately comparable, besides comparing the incidence and the duration of complete remissions, seems important in an attempt to relate the survival also to the histologic expressions as well as to the extent and type of organ involvement. Cumulatively, all these data will contribute to a better definition of cure (*47*) of Hodgkin's disease.

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SUMMARY OF INFORMAL DISCUSSION ON CURRENT STATUS OF CLINICAL TRIALS

M. Tubiana remarked that he was very much impressed by the high quality of the clinical research carried on in the advanced stages of Hodgkin's disease, leading to very high survivals. His second remark was much less optimistic. He also believed we are entering a state of confusion because of staging. Some of the authors were giving their data in terms of pathologic staging, others referred to the clinical staging, and others used a mixture of the two. If we are not careful, we will soon reach a point where nobody will understand anybody. It must be emphasized that use of pathologic stage instead of clinical stage improves a result by introducing 2 sources of bias. It takes out of the less advanced stages the worst cases and introduces into the more advanced stages some early cases. Therefore, he urged those who presented their data with regard to pathologic stage to introduce also in their published data some information with regard to clinical stage so that comparison will be possible. In particular, it would be a pity if it were impossible to compare the data from Stanford University, which are so important, with the data from other clinical trials.

H. S. Kaplan agreed with Doctor Tubiana about the hazard of confusion in staging terminology. He also questioned the arguments made by Doctor Bonadonna for using radiotherapy after, rather than before, chemotherapy. He did not really understand that rationale. He would grant that there is a high frequency of occult sites of involvement in patients with advanced disease, especially the Stage III-B cases, and that many of those occult sites will be extralymphatic, but he did not see that that makes an argument for treating the microscopic occult sites first and delaying definitive radiotherapeutic treatment until last for the areas known to be involved. At present, many groups throughout the world have corroborated the earlier data from Stanford University indicating that the "complete remission rate" in each involved lymph-node chain treated with adequate doses of radiotherapy, in the range of 4000 rads/4 weeks, is approximately 99%. There are no chemotherapeutic data which approach a 100% control rate for known involved lymph-node chains. When one starts with the chemotherapy, one runs the risk that it will not be possible to give the full dose of radiotherapy to some of the patients to whom one has given combination chemotherapy previously. In that case, the use of the most definitive agent for the known disease is jeopardized to give as yet unproved treatment prophylactically for disease which may or may not be present in the other sites. That does not seem a logical approach.

Furthermore, it would appear from the experience thus far at Stanford University versus that at the National Cancer Institute in Bethesda, using the 2 different sequences, and with the same 4 drugs given in the same way, that it has not been possible to use radiotherapy after MOPP chemotherapy (mechlorethamine, vincristine, procarbazine, and prednisone combined) at Bethesda with any real consistency, whereas in the Stanford experience thus far only 4 of 50 patients failed to complete 6 full cycles of MOPP after having completed total lymphoid radiotherapy. Accordingly, he believed that, if one is going to use both treatments, the arguments weigh in favor of using the radiotherapy first. **Tubiana** concurred, saying that his group found that giving chemotherapy first, before radiotherapy, makes it difficult to give radiotherapy in about 20–25% of the patients. **G. Bonadonna** replied that, if a certain number of these Stage II-B and III-B patients are going to have a relapse, it is probably because they have disease outside of the lymphatic system. The patient is not going to die because he has uncontrolled disease in the mediastinum or in the para-aortic nodes but because of the disease elsewhere, outside the lymphatic system.

Tubiana pointed out that the amount of treatment one has to give is proportional to the size of involved organ or tissue, so it is better to treat first the big mass than the small one. **E. Frei** said that, if one is going to use 6 cycles of MOPP or something comparable to that and also total-nodal radiotherapy, it has been clearly demonstrated that the sequence of radiotherapy followed by chemotherapy is much better tolerated and probably superior. However, there is a circumstance where radiotherapy can follow chemotherapy and that is in patients with Stage IV-B disease where his group demonstrated that, for those patients who have a relapse, there is an excellent correlation between the site of initial relapse and the site of major pretreatment involvement. If one plots pretreatment involvement sites by descending order of mass and correlates that with the first site of relapse, the correlation is exact in most cases. This is not surprising, if one accepts the view that we are not producing cellular cure by chemotherapy. As a result of this analysis of a previous study, his group has started a study involving chemotherapy, using a modification of the MOPP program, followed by radiotherapy, not total-nodal radiotherapy but rather radiotherapy delivered to the major (most massive) sites of primary pretreatment involvement. **S. A. Rosenberg** added that the Stanford group could deliver involved field and even total-nodal radiotherapy quite effectively after 2 or more cycles of MOPP in patients with Stage IV disease.

P. P. Carbone commented that there is another situation in which chemotherapy might, in fact, replace radiotherapy and that is in the developing countries where radiotherapy facilities are not available. In Uganda, the National Cancer Institute group have had the opportunity to treat essentially all patients with Hodgkin's disease, after appropriate clinical staging, with combination chemotherapy. Although the distribution of the disease is different in Uganda than in the United States, they do see some patients with Stage I and II disease and early Stage III disease who have been treated with chemotherapy and have done extremely well. Thus, we will have an opportunity at least to see, in this situation where there are no adequate radiotherapy facilities within hundreds of miles of the area, what will happen with chemotherapy alone.

Part 2. Panel Discussion: Types of Clinical Trials Currently Needed

Chairman: M. Tubiana

INTRODUCTION

We have heard about the present status of the treatment of Hodgkin's disease. We will now ask the panel to discuss the new goals for the future. It seems to me that there are at least 2 types of questions and, therefore, 2 types of investigation that are needed. One is to better evaluate the risks of some treatment procedures and to try to reduce unnecessary treatment. With modern types of treatment, the prognosis is excellent, but probably many patients are overtreated and a few are undertreated. Overtreatment carries genetic and somatic hazards. How can we reduce the proportion of patients who are overtreated? I can suggest 3 possible ways: 1) Are there some types of patients in whom relapses occurring after limited treatment, when properly treated, would not reduce survival? 2) Is it possible to rely on prognostic factors and patterns of presentation for choosing therapy? 3) Is it possible to rely on new types of examinations for the detection of residual or occult disease? The second question, that we shall discuss first, is how can we improve the present results for those stages and types of disease for which the results are not yet satisfactory.

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Invited Discussion: The British National Lymphoma Investigation[1,2]

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THE BRITISH National Lymphoma Investigation, in which colleagues from 17 centers are collaborating, was started in February 1970, after more than 2 years of planning and discussion. The aim of this investigation is to find out the *minimum* amount of treatment that would cure the *maximum* number of patients with Hodgkin's disease. Our group considered that, although there might well be a place for complicated comparative trials, there was a need for comparison of less sophisticated and easily applicable methods of treatment.

Our collaborators record *all* new patients seen by them. In this way, we avoid bias and the exclusion of patients for personal reasons. Patients are accepted into the investigation if our pathology panel agrees that the biopsy confirms Hodgkin's diseases which can be placed satisfactorily into one of the generally accepted 4 pathologic subgroups and when the disease does not appear to *originate* in one of the less common extranodal sites, e.g., the stomach, intestine, or extradural region. Patients are also excluded when they have had more than an agreed minimum amount of previous treatment, when certain mandatory investigations have not been completed, when the patient is medically unfit for radical treatment, or when adequate follow-up is geographically impossible.

Of the 365 patients submitted by January 1972, 215 were accepted into the investigation and randomized for treatment; many more have not yet completed investigations. Laparotomy is not a mandatory investigation. Some collaborators laparotomize all patients in whom there is no medical contraindication, and others do not. Patients are staged either clinically, or pathologically, when laparotomy has been performed. Before randomization, all patients are subdivided into groups depending on the extent of the disease, presence of systemic manifestations, age and sex, and the histologic appearance of the lymph node.

In patients with disease apparently limited to one side of the diaphragm (Stage I–II, A), we are comparing relatively localized irradiation of the involved regions with prophylactic irradiation including the uninvolved regions adjacent to the affected areas. When systemic manifestations are present (Stage I–II, B), the prognosis is worse. We have therefore accepted that wider field irradiation is justifiable despite the theoretically greater risk of long-term complications. Prophylactic treatment is extended in this group to total-node irradiation. So far, there is no obvious difference between the groups of patients given either local radiotherapy or wide-field radiotherapy, whether systemic manifestations are present or absent. We are surprised that the results are so similar in the 2 treatment groups of patients with systemic manifestations. It may be too early to detect a significant difference, but this may become apparent with longer follow-up.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] This study is supported by the Aitchison Charitable Trust, the Batchworth Trust, the Cancer Research Campaign, the J. S. Fraser Trust Fund, the New Court Charitable Trust, the Peggy Russell Memorial Fund, and Eli Lilly & Co., Ltd.

[3] The collaborating clinicians are working at United Birmingham Hospitals, Birmingham; Addenbrookes Hospital, Cambridge; Velindre Hospital, Cardiff; Coventry & Warwickshire Hospital, Coventry; Harefield Hospital, Harefield; Cookridge Hospital, Leeds; Clatterbridge Hospital, Liverpool; Hammersmith Hospital, London Hospital, Middlesex Hospital, and Royal Marsden Hospital, London; Mount Vernon Hospital, Northwood; Freedom Fields Hospital, Plymouth; Royal Southants Hospital, Southampton; and Royal Cornwall Hospital, Truro.

When Hodgkin's disease is demonstrated both above and below the diaphragm, but is still localized to the lymph nodes or spleen (Stage III, A and B), our group considered that it was necessary to compare total-node irradiation as advocated and carried out so admirably by H. S. Kaplan, with the best available cytotoxic drugs. We had no doubt that combination therapy, as advocated by V. T. DeVita and P. P. Carbone, offered the best possibility of long-term chemotherapeutic control. Our comparison was therefore between these 2 methods of treatment. After only 2 years, numbers are too small to be of statistical significance, but there have been no deaths from Hodgkin's disease in either of the 2 treatment groups when systemic symptoms were *absent.* When systemic symptoms were *present,* chemotherapy appears to be achieving better control. This may be due to the fact that the presence of systemic manifestations indicates hepatic involvement which is difficult to demonstrate, even after laparotomy and liver biopsy.

When the disease extends beyond the lymph nodes to involve extralymphatic structures, such as the liver, bone marrow, lung, or skin (Stage IV, A and B), cytotoxic drugs offer the best method of control. Our group first compared the effect of a pulsed single agent with pulsed quadruple therapy of the MOPP type (mechlorethamine, vincristine, procarbazine, and prednisone combined). The single agent used was chlorambucil. After a few months, the patients treated by the pulsed, single agent were less well controlled and developed more marked hematopoietic depression than those treated by pulsed quadruple therapy. This investigation was therefore stopped. Further follow-up of these patients confirmed that the results with the 4 agents were superior to those with the single agent. These results will be published in the future.

The group then decided to compare the effects of pulsed quadruple therapy using nitrogen mustard, vincristine, procarbazine, and prednisone with a regimen which differed only in that the prednisone was omitted entirely. After the first 8 months, there was no difference between these 2 groups of patients. Control was as efficient, and toxicity equally limited with both regimens. However, long-term assessment of these groups is necessary before we can decide whether the addition of prednisone is advantageous. With either method, we have achieved a 71% remission rate.

SUMMARY OF INFORMAL DISCUSSION BY THE PANEL

V. T. DeVita expressed the view that much of the problem in the current chemotherapy trials with combinations of drugs rests on two bases: 1) the input of patients and 2) the definition of what we mean by complete response. We are having a great deal of difficulty not only in speaking the language of staging, but also in randomly allocating patients within given studies according to prognostic indicators. For instance, one must take into account that patients come to such studies frequently after having failed in a radiotherapy trial. One has to consider that the prognosis of the patient who began with localized disease and has now eventuated into advanced disease differs from that of the patient who presents initially as a Stage IV. If one does not consider the balance of these 2 types of patients, one can introduce a therapeutic bias into the study.

One other area of concern is that many groups have tended to delete the option of an unmaintained control group who receive no therapy after achieving a complete remission. This is based on a belief that maintenance therapy is better than no maintenance therapy. It is not clear that this has been accurately determined. If the criteria for complete remission were uniform, we might find that maintenance drug therapy is not better than no maintenance. In fact, we should consider the possibility, if we worry about the ethics of the situation, that this approach may actually be harmful. Continued combination chemotherapy in excess of say a year might be very difficult for patients to tolerate. Each study should now continue to include a group of patients who receive no further therapy after induction of remission. This also serves as a cross-check on the complete remission rate. Several studies now in the literature, or on their way, show a very high complete remission rate, but a very short duration of complete remission. In other words, the iceberg has been pushed just below the surface. The minute therapy is stopped the disease pops back. To serve as a cross-check on the validity and the usefulness of the high complete remission rates, one needs a group of patients who were left without any further therapy. Then one could see if each combination is comparable in terms of remission rate, remission duration, and ultimately survival. The use of postremission unmaintained control groups, the development of criteria for randomization and stratification of patients, and the definition of uniform criteria for complete remission would greatly improve results in the future for trials of combination chemotherapy, as well as the direct comparison of one combination to another or of one therapy to another.

S. A. Rosenberg commented that, with respect to improving results, there are 2 major areas occupying much of our attention. One is how best to use the chemotherapy being developed, specifically maintenance chemotherapy, to prolong the remissions which have been achieved. Doctor Rosenberg cited an analysis that Doctor Moore had done at Stanford University of the use of MOPP (mechlorethamine, vincristine, procarbazine, and prednisone combined) in patients other than the protocol group that Doctor Kaplan had described. In contrast to Doctor McElwain's maintenance treatment, these patients have received just 6 cycles of MOPP, with no maintenance therapy thereafter. Doctor Moore had attempted to break down what variables were responsible for, or associated with, relapse. There is some difference as to whether there are systemic symptoms at the time of treatment and the frequency of complete remission. There is also a marked difference as far as age is concerned in helping to select out the groups that have a rapid relapse versus groups that can remain in remission without further chemotherapy. Moreover, these results were completely different from those of Doctor McElwain with respect to prior treatment, in that the groups that had no prior therapy have rapidly and almost completely relapsed (90% at 16 months). This merely emphasizes that the groups that one accepts for such chemotherapy will differ in different institutions. Perhaps those that the Stanford group are willing to treat with chemotherapy alone are a much more advanced group than might occur in other institutions. The Stanford patients with no prior therapy who were given MOPP alone have all had very advanced Ann Arbor Stage IV disease. There are no III-B's, for example, and all cases are pathologically staged.

The question of maintenance chemotherapy is on all of our minds, and it will have to be settled by studies which are done very carefully. In that regard, he wondered whether one has to have a no-treatment group. He was attracted to the idea of using 2 different kinds of maintenance therapy. However, that runs the risk that they will both give the same result and then one will not be able to conclude that there is an advantage of maintenance over none. If one type of maintenance is better than the other, then might one conclude without a no-maintenance group that there is advantage in maintenance therapy?

The other point is that one needs approaches for the advanced Stage IV's which are better than chemotherapy alone. At least the results are so poor with chemotherapy alone that we have to consider using radiotherapy either to those sites which are the major sites of disease or those sites which are residual after chemotherapy. The approach of the Stanford group has been to take the advanced Stage IV's, give them a split course (2 or 3 cycles) of the MOPP program, then to proceed with total-nodal radiotherapy, and finally complete the remaining cycles of MOPP in an effort to control the disease with the best of both modalities. Data are not yet adequate except for anecdotal examples of 3 or 4 patients in whom this type of treatment has been possible and who have been relapse-free for a few years.

E. Frei pointed out that, with the tremendous advances made in staging, radiotherapy, and chemotherapy, the numbers of variables we now deal with are quite enormous. Substantial attention must be given to the problem of experimental design. The simpler the experimental design, the more likely you are to get an answer. The more complex the experimental design, the more questions you can address yourself to, but you risk answering none of them. Doctor Frei believed that, in the current stage of Hodgkin's disease studies, we are at risk of that kind of experimental design.

In chemotherapy, in addition to the component drugs of the MOPP program which have improved the prognosis of advanced disease very substantially, and perhaps cured some patients, we now have several additional drugs, some of which may be equally effective. The experience of Doctor Frei and his group with Adriamycin in patients with advanced disease is comparable to that of Doctor Bonadonna. In their sample of patients, Adriamycin is yielding about a 70% response rate in advanced or refractory patients with Hodgkin's disease, approximately 20% of whom have had complete remission. This compares favorably with the best of known agents, and perhaps when moved up front it will be even better. Bleomycin is also good, though complete remissions are relatively rare. In their experience, Bleomycin and dimethylimidazole carboxamide in patients with advanced disease have each produced about a 60% response rate (objective response being defined as complete and partial remissions).

The question is how to put this all together to maximize benefit to the patient. A great deal of attention must be paid to scheduling. **E. Hersh** has demonstrated that intermittent treatment is probably better than continuous treatment in terms of the overall immunosuppressive effect. There is ample experimental evidence, at least for more rapidly growing tumors, that intermittent treatment with cell-cycle-specific agents, and perhaps with non-cell-cycle-specific agents as well, is superior. Another important feature is the intimate time relationship [illegible] the scheduling of drug administration. His group has a program that involves pretreatment with vincristine, which synchronizes the cells in metaphase, followed by treatment with Bleomycin at 6–12 hours—that is, following synchronization. Bleomycin has been demonstrated to maximally reduce viability of synchronized cells in metaphase or in late G2, so what we know of the cell cycle activity of the individual cells can be exploited in scheduling.

One of the most crucial questions that comes up with respect to chemotherapy relates to how long one should treat. When can one stop treatment? **Frei** presented a study which was directly addressed to this problem. This study, initiated about 4½ years ago in the Southwest group, involved patients with disseminated disease who were treated with 6 cycles of MOPP. If they did not respond, they went off treatment. If they had partial remission, they were continued on MOPP. If they entered complete remission either directly or through continued treatment, one group was randomized to no further treatment, substantially the same as in the initial treatment program of the National Cancer Institute. The other group got 18 months of MOPP therapy at 2-month intervals. This was after it became pretty clear that one could not continue MOPP at 1-month intervals, but if one allowed a 2-month interval there was little cumulative toxicity. Most of these patients in fact got through 18 months without cumulative toxicity.

The median time to complete remission for those patients entering complete remission on MOPP is approximately 3–4 months. If one accepts some simple assumptions, such as first-order kinetic effects for antitumor agents and that the number of tumor cells was about 11–12 orders of magnitude, then 6 months of treatment should be inadequate to eradicate all of the tumor cells, whereas the projected time would be in the neighborhood of 12–18 months. If 6 months of monthly MOPP were followed by 18 months or 2 years of MOPP every 2 months, that would cover that time period and, hopefully, might have a major effect on the disease.

A breakdown of the patients by prognostic category reveals that 60% were in Stage IV. Otherwise, it was a pretty random sample. In terms of complete remission rates, Stage IV-B patients did not do as well as the other 3 stages, and the patients with major prior chemotherapy and radiotherapy did not do as well as the others. **H. S. Kaplan** asked which staging system was used. **Frei** replied that the Rye staging system had been used. **Kaplan** then asked whether, in that case, some of the Stage IV's would not have been II-E's or III-E's in the Ann Arbor classification. **Frei** replied that there were very few such cases. The study was almost all based on clinical staging. Case accrual for this study started about 4 years ago and was stopped about 2 years ago. **Kaplan** asked whether these stage allocations had been made at the time of relapse, rather than initially. **Frei** stated that these were the stages at the time of the start of study. **Kaplan** pointed out that, since some of them had had prior therapy, they must have had an earlier stage.

Frei concurred and said that his comment referred to the stage at the time patients were started on this MOPP program. Continuing his presentation, he showed data indicating that, when the duration of complete remission is plotted from time of randomization for the unmaintained patients, the risk of relapse decreases with time and that, after about 3 years, when the numbers of patients are small, it appears to plateau at approximately 45%. This corresponds reasonably closely to the results observed at the National Cancer Institute and by others. However, if one looks at the maintained patients, there is a substantially better result. Comparison with the unmaintained group yields a P value of 0.02 so there is a significant difference. At this point in time, maintenance therapy was discontinued. Although there was a decreased risk of relapse during the period of maintenance treatment as compared to the unmaintained patients, some of the patients were perhaps just barely held in remission because there was an early increase in numbers of relapses thereafter.

They had analyzed in detail some 15 patients who had relapses and on whom they had definitive data as to the distribution of the disease before the MOPP study in terms of magnitude or bulk of disease in different sites. When these are compared with the site or sites of relapse by order of appearance, the correlation is exact in 9 of the 15 and good in the remainder. There was evidence, albeit not conclusive, that if one took the total bulk of the disease and tried to relate it to the duration of remission, there was an inverse correlation. The more disease before treatment, the shorter the remission. Certainly the site of initial relapse does correlate very well with the major site of pretreatment involvement. Accordingly, in their current protocol, which has several variations for remission induction with combination chemotherapy, one of the maintenance arms does involve radiotherapy to the major pretreatment site(s) of involvement after the patient has entered complete remission.

Finally, they have data on the effect of remission on survival. For those patients who do not enter complete remission on MOPP, the median survival is short. It is not particularly good for patients who enter partial remission. The complete remitters, however, do very well. Among those randomized to maintenance versus those remaining unmaintained, there is a slight difference in favor of the maintained group, but this is not significant. About 80% of these patients are still in complete remission beyond the 3-year mark.

DeVita suggested that the use of maintenance therapy comes about from our inability as chemotherapists to come to grips with the possibility that we might occasionally cure a patient with disseminated disease. This is a problem that other specialties do not have to deal with. What we need to consider is continued reinduction or retreatment in patients while in remission, but we tend to back off from the intensive chemotherapy programs that we have. It may be that at some point maintenance becomes totally ineffective. We should consider developing new programs and then cross-over and treat patients who are in remission, regardless of disease, with the new program, whether or not it is somewhat different or entirely different from the previous program, to see if we could prevent more patients from relapsing. In Doctor van der Werf-Messing's study, it looks as if the prolongation of remission, while valuable for the patient in terms of prolonged lifespan, is not going to last, and that eventually those 2 curves are coming together, and the long-term survival may be no different in both groups.

B. van der Werf-Messing replied that those patients who completed a full 2 years of maintenance chemotherapy with vinblastine did extremely well and may have been permanently cured.

M. Tubiana asked Doctor DeVita what he meant by maintenance therapy. How long is the maintenance therapy continued? **DeVita** replied that, for most single drug programs in the past, it has been for the duration of the patient's life. That was rather easy because it was of rather a short duration, <2 years. Now that patients are living longer and we are using more intensive chemotherapy programs, we are having difficulty giving maintenance therapy for the whole duration of the patient's life. All of the studies to date have employed a finite period if they used combination chemotherapy for maintenance. DeVita believed that the answers to the questions raised about the proper duration to give "maintenance therapy" are totally unknown—perhaps it is somewhere in the range that Doctor Frei has suggested—about 2 years of intensive therapy may be required.

G. Bonadonna added that his group had not planned so far. Although they do not have a definite program for the total time period they will treat their patients, they would not exceed 2 years. But he did not have concrete results to indicate whether such patients should be treated for >2 years or for <2 years.

To have comparable results in the different groups of patients, we should pay attention to how we randomize these cases. Since the variables are different—e.g., age, sex, and histologic expression—in their protocol, they take both the stage and the histologic expression into consideration for comparison of the results. In other words, the cases are stratified within stages into the 4 histologic types, thus forming subgroups which can be compared at the end of the study. They assign to either maintenance or no maintenance the same number per group of each histologic type. This takes place also for Stage IV, but the main variable for Stage IV is not only the histologic expression but also the extent of organ involvement. There are different types of Stage IV, and they should be compared with the same type whenever possible. Probably liver involvement does not have the same prognosis as bone involvement. Their randomization takes these variables into consideration and this probably helps in comparing the results.

Tubiana commented that, in some of the older studies done for breast cancer, the investigators took into consideration the so-called dominant organ involvement. He thought this should also be considered in Hodgkin's disease.

DeVita expressed the view that we really need some sort of marker of residual tumor, which would be able to circumvent questions of predominant organ involvement. If one could measure the residual tumor in some way, perhaps by some specific antigen from the tumor, this would be an important guide to therapy. He looked forward to being able to have something like this in the next 5 or 10 years.

Tubiana summarized the several types of new treatment suggested by the Panel: 1) maintenance therapy for about 2 years, 2) a better combination of radiotherapy and chemotherapy, 3) either new drugs or new types of combinations of scheduling, etc. He then asked if anybody in the audience wanted to comment on these types of new clinical trials or to suggest any other new types. **Kaplan** questioned whether, if a combination of chemotherapy were used in conjunction with radiotherapy in the earlier stages, the dose of radiotherapy could be lower, at least for areas not massively involved; therefore, in areas that are treated prophylactically with radiotherapy, would it be possible to use substantially smaller doses to minimize the hazard of late radiation complications and improve tolerance? **Tubiana** asked whether it was possible to develop ways to select the type of patient who will respond better to one type of chemotherapy than to another by in vitro testing or by studying the kinetics of the tumor or some other laboratory test.

P. P. Carbone commented that it is very possible that what we call late recurrence is a de novo reinduction of the disease by its causative agent. There is some information in Uganda among patients with Burkitt's lymphoma that the early relapses are much different than the late relapses. That one may possibly have a reinduction can explain why reoccurrences are occurring late.

Kaplan said he was concerned when Doctor Bonadonna was referring to the problems of evaluation of Stage IV cases. People are clearly using the term Stage IV in different ways, which will get us into difficulty. Some of the Stage IV's are Ann Arbor Stage IV's involving primarily bilateral lung or bone marrow or liver, whereas others are Rye Stage IV's (Ann Arbor II-E's or III-E's) with perhaps sternal or other solitary bone involvement, or extradural or pericardial involvement, all of which have been shown to be highly treatable with radiotherapy alone and thus really do not qualify as Stage IV's. In this context, he was struck by the fact that Doctor DeVita's data, which included some Stage IV's, show a low relapse rate over a considerable time, whereas the data that Doctor Rosenberg had referred to, on a series of cases which were called pure Stage IV's by the Ann Arbor criteria, all of which had either bone marrow or bilateral lung disease, had a 90% relapse rate at 16 months after treatment with Doctor DeVita's MOPP quadruple drug program. This makes one wonder how many of the Stage IV's in the earlier study at the National Cancer Institute were Stage IV's by present (Ann Arbor) criteria. If the Stage IV data of Stanford University are correct, they would suggest that MOPP has failed to eradicate disease in areas inaccessible to the radiotherapist.

DeVita replied that there were 2 cases that would have been called II-E now. He believed that, with all due respect to the results of radiotherapy, in that kind of patient chemotherapy does equally well and perhaps better than radiotherapy, though this opinion has never been tested in a randomized trial. Many of the other patients had quite advanced disease. In fact, some patients who were extremely ill with multiorgan involvement have remained free of disease for 4, 5, and 6 years. Doctor DeVita felt that the largest part of the discrepancy is perhaps based on the selection of more advanced patients.

Tubiana concurred in the difficulty of comparing different types of Stage IV cases, those with different organs involved, those with previous treatment and relapse versus those previously untreated, etc. Here again he would urge everybody to make a distinction when presenting data, because a treatment may be very appropriate for new patients not treated before and not appropriate for previously treated patients.

DeVita added a comment about the difference in combination chemotherapy programs. His group had initiated studies with the MOPP combination and obtained the results which they reported. Now, they frequently hear that some other group used this combination and obtained a different result. He could think of at least one situation in which one group has been unable to get a significant number of complete remissions in previously untreated patients. When one analyzes what they did, although they reported that they had given MOPP, they had changed it very significantly. At the National Cancer Institute, they have never seen a patient go into remission, for instance, who has had evidence of regrowth of his tumors between cycles. Therefore, physicians who use the MOPP program but spread out the intervals between cycles, waiting for blood counts to return totally to normal, risk having patients go into relapse between cycles. Many physicians are very reluctant to give the full doses of the 4 compounds, and many have omitted doses of drugs entirely instead of scaling them down. Therefore, one has to analyze the data in detail to see if any of these types of biases is present. **Rosenberg** said that his group had achieved the same complete remission rate that Doctor DeVita reported, and especially in the no-prior-treatment group he had given comparable doses of drugs so he does not think that explains the difference. Instead, he believed that case selection accounts for the discrepancy.

Frei pointed out that the tactics and the strategy of chemotherapy have to be emphasized. As chemotherapy becomes more complex, it becomes increasingly evident that groups using the same protocol, or institutes using the same protocol, may, in fact, come up with different results. The Southwest Group has analyzed this now for Hodgkin's disease on their combination chemotherapy program and also for acute leukemia in adults on combination chemotherapy programs. If one compares those institutes which contribute the largest number of cases with those institutes contributing relatively small numbers of cases, there is always a difference in results in favor of the former, and sometimes the difference may be very significant. This is not to demean the smaller institutes. It is just to indicate that experience with the program increases the flexibility and efficiency of its use. Experience indicates that one does have to resume treatment at monthly intervals; one cannot wait for longer intervals of time. One must resume treatment at a certain dose level, etc. These details all come under the tactics of chemotherapy and become increasingly crucial as chemotherapy becomes more complex. These are very subtle distinctions that one does not see unless one has all the data. **Tubiana** agreed that this is a very pertinent comment. He and his colleagues have the same feeling in the European trial about the results obtained in the large and the small centers.

H. Rappaport pointed out that, in the new clinical trial of the European Organization for Research on Treatment of Cancer (E.O.R.T.C.), the histologic type of Hodgkin's disease will in part determine the treatment assignment.

This places a very heavy responsibility on the pathologist, and, since all pathologists are human and therefore fallible, it raises a question about the validity of omitting chemotherapy in cases of nodular sclerosis.

Tubiana replied that one of the difficult problems about it is the course of the disease is not the same in the patient with nodular sclerosis and the patient with mixed cellularity. The difference may be related either to the course of the disease or to the chemosensitivity. This is a topic which can be discussed better after the other data from the E.O.R.T.C. have been presented.

He then asked the panel to turn to the second part of the discussion. How do we handle the treatment of patients with less advanced disease, some of whom are perhaps overtreated with the present type of treatment?

Rosenberg discussed the problem of reducing the amount of treatment in patients with less advanced disease. To oversimplify the currently recommended therapy for Hodgkin's disease, it can be stated that patients in Stages I and II, whether A or B, no matter what histologic type, and patients in Stage III, particularly those without systemic symptoms, should have total-nodal radiotherapy to high doses of the order of 4000 rads in 4 weeks per field. Patients with more advanced disease should have aggressive chemotherapy. In some centers, Stage III-B would fall into the radiotherapy group.

If this is the current state, then a great deal of work has to be done to try to analyze whether there are many patients being overtreated. Some obviously are being overtreated because there are very good results with different forms of therapy for different groups of patients. One must be careful to ask questions which are not trivial. A trivial question is one which is really not worth answering, especially in terms of the investment of time and energy required to answer it. For example, if one merely wanted to exclude the axillary region during total-nodal radiotherapy it would take a long time to prove whether it is valuable or not and, since the morbidity that one finds with this kind of treatment is not great, that would be a trivial question. But there are other questions which at least should be asked. Has aggressive chemotherapy been used in the least advantageous situation, in patients with far-advanced and often recurrent disease, thus making it difficult to answer whether it has a better potential? This question cannot be answered rapidly; it must be done in steps, gradually shifting toward a more favorable clinical setting. One of them may be, as Doctor Kaplan mentioned, to gradually reduce the radiotherapy either to known or unknown sites and then complement this with combination chemotherapy to see whether the chemotherapy can totally eradicate the microscopic residual disease left after the smaller dose of radiotherapy. That approach presumes that the chemotherapy is less morbid than the high-dose radiotherapy.

The other possibility we should consider is whether combination chemotherapy can substitute completely for prophylactic radiotherapy in patients with favorable disease. The Stanford University group has embarked upon a study to try to answer that particular question. This experimental design also tries to consider the next major question which we have to approach, i.e., whether histologic type should be used in the selection of treatment. What has been done at Stanford University is to distinguish the favorable groups of patients from the unfavorable groups with respect to extent of disease, absence of systemic symptoms, certain sites of lymph-node involvement, certain histologic types, etc. The favorable group may be overtreated by total-nodal radiotherapy; whereas the unfavorable group, involving certain sites, especially the left neck and the para-aortic nodes, and the more unfavorable histologies, may justify such aggressive radiotherapy. Two new protocols, the R-1 and the K-1, have been established. The favorable cases are randomized within the R-1 protocol. One group receives limited extended-field radiotherapy only; the other receives high-dose radiotherapy limited to the site (s) of involvement, followed by 6 cycles of MOPP to try to determine whether the MOPP can substitute for the prophylactic irradiation of apparently uninvolved lymph-node chains and, therefore, reduce late radiation morbidity. In the more unfavorable cases, both groups are being treated with subtotal radiotherapy (omitting the pelvis in supradiaphragmatic cases) followed by MOPP chemotherapy in half the patients. That is the appropriate kind of study, giving less radiotherapy and at the same time testing the chemotherapy in the best possible setting. He did not think we are yet ready to use chemotherapy alone in these settings, but one should get results from this generation of studies and then re-evaluate.

Some other questions have already been alluded to, such as the optimal timing of aggressive radiotherapy and combination chemotherapy. We have all wondered, if one waits for relapse and then applies the next measure of treatment, whether in the long run survival will be diminished. Retrospective analyses indicate that patients who have relapses after radiotherapy do not do well. They are apparently at a greater risk for continuous loss of life, and it has not seemed justified to defer treatment that might prevent relapse. But now that combination chemotherapy is available as a sequence following radiotherapy, it seems quite appropriate to set up a randomized study which uses the chemotherapy either immediately after radiotherapy or at the time of the first relapse. He would predict that it would be better to use it immediately after radiotherapy, when the amount of tumor is the least, but that could be tested.

There is another important question very difficult to approach. What is the effect of splenectomy per se? Is this a trivial question or not? Does the splenectomy change the course of the disease, either favorably or unfavorably, and, therefore, justify the operative morbidity or the possible long-term complications of splenectomy? If one could conceive of the appropriate study to test that as the variable, it would be of great interest to many physicians. On the other hand, it is very difficult to imagine how to do the study correctly. The stages will often change after the laparotomy and splenectomy and then the therapy will be different.

His final question was: "What should one do with the very young patient with Hodgkin's disease? The patients under the age of 12, or perhaps under the age of 10." We need to consider the long-term effects on growth of both radiotherapy and chemotherapy and also the effect of splenectomy in this group of patients. We probably should be willing to give less radiotherapy, cover it with chemotherapy, and perhaps avoid splenectomy in this group. But that in itself would require study.

Tubiana commented with regard to the children that the E.O.R.T.C. groups have quite a lot of children in their files. Children do not seem to respond so differently from adults as to justify a different type of treatment. **Rosenberg** asked whether he meant they were no different as far as their disease or their complications of therapy were concerned. **Tubiana** replied that he considered this true for both. Furthermore, he pointed out that what Doctor Rosenberg said implies that radiotherapy is more harmful for children than chemotherapy, which remains to be proved.

Rosenberg said that, in youngsters who have had the opportunity to grow for 5 or more years, radiation to a dose of 4000 rads has produced marked growth problems. If we have more and more success in treating these patients, many of them will be surviving through the growth period. Even the mantle field alone has changed the configuration of some patients. If they received total-nodal radiation, he believed it would produce adults with long extremities and a short trunk. Is there a dose of radiotherapy that will minimize that and can it be supplemented effectively with additional chemotherapy?

Kaplan commented that he had treated 2 children with Stage III_S-B disease with 1500 rads to all sites—i.e., total-lymphoid radiotherapy but to a much lower radiation dose—followed by 6 cycles of MOPP. One child was 21 months old and the other, 4½ years old. Both children are alive and relapse-free at 2 years and have normal growth.

Tubiana added that his group has about 20 or 30 children. **Kaplan** asked how many were under 5 years of age. **Tubiana** replied that he did not know for sure, but perhaps 5. Most of them have been treated with radiotherapy with or without added Velban to the usual dose levels. Radiotherapy does not seem to be very harmful. **Rosenberg** asked whether the possible harmful effects of chemotherapy on growth and development in children had ever been studied. **Tubiana** agreed that data are insufficient on this point.

DeVita claimed that the scattered radiation would be enough to sterilize a good number of children. This was sharply denied by **Tubiana. Rosenberg** stated that one has to examine spermatogenesis in adults previously treated with radiotherapy during childhood. Some radiotherapists have estimated that, during treatment of the pelvis to 4000 rads, a scattered dose as high as 400–800 rads may be given to the testes, so the possibility of significant late damage to spermatogenesis is worth considering. **Dr. van der Werf-Messing** commented that perhaps the discrepancy between Doctor Tubiana's views and those of Doctor Rosenberg related to the fact that the children in the E.O.R.T.C. study did not get total-nodal radiotherapy; they just got radiation to one side of the diaphragm. Therefore, one cannot compare the 2 groups.

S. Hellman commented that perhaps the most important point in Doctor Rosenberg's proposed study is that he was not going to irradiate the pelvis in any of the "unfavorable" Stage I or II cases. Although we have all talked about inverted-Y fields, we should separate the para-aortic nodes and the pelvic (low iliac) nodes. If he had understood Doctors Hutchison's and Nickson's studies, they were irradiating patients to the para-aortic nodes and the mantle, but not to the pelvis in Stages I and II. It would be of interest to know their site of recurrence in those that fail. If it is in the pelvis, then one could strongly argue that we would be much better off irradiating the pelvis than adding chemotherapy. **G. B. Hutchison** said he did not have the data on this specific point with him at the Symposium.

Rosenberg wondered if it is considered trivial by the radiotherapists whether the dose for Hodgkin's disease is 4400 rads or 3500 rads. It seemed to him that that might be right at the border of much more harm to normal tissues if you go beyond that range, but his colleague says that is trivial. **J. J. Nickson** commented that it may or may not be trivial. The national cooperative clinical trial will have a pretty fair answer to it, at least in one respect. It should be possible to compare the Stanford University complication rate in the earlier protocols, in which the fields were similar but the dose was 4400 rads, with the national trial data, in which most of the participants had used doses of about 3500 rads and only rarely exceeded 4000 rads.

Frei suggested that this is perhaps another question that has already been answered by earlier studies. He recalled that, in some of the earlier studies by Doctor Peters, the evident disease was irradiated with 4000 or 4400 rads but the prophylactic areas were irradiated with substantially lower doses. In that group, are there more recurrences in the prophylactic area as compared to those that did not receive prophylactic irradiation? **Kaplan** replied that the difference in dose was very great. Doctor Peters had used ≦1500 rads in the prophylactic areas, a much lower dose than the 4400 rads used at Stanford University. **Rosenberg** did not clarify in his question whether he was referring to the dose to the diseased areas or the dose to the "prophylactic" areas. **Rosenberg** replied that he was interested in both situations, and whether the dose has to be 4400 rads. **Kaplan** then commented that data were inadequate on the dose required to eradicate occult, microscopic disease and thus that perhaps the dose to prophylactic areas may not be a trivial question. **van der Werf-Messing** stated that, in her experience, a radiation dose of 4000 rads in 4 weeks had been associated with a 4% recurrence rate, mostly in marginal sites, and no significant complications to date.

Tubiana then expressed appreciation to the panelists and the audience and declared the session adjourned.

Part 3. Complications

Chairman: G. Hamilton Fairley

INTRODUCTION

This session begins with a discussion of complications. Because the complications of radiotherapy and chemotherapy are so different from those of exploratory laparotomy, we will have a short discussion after each section and not a general discussion at the end. I think that the complications we are now beginning to see, particularly with intensive radiotherapy and chemotherapy, are very interesting and on some occasions rather alarming. However, to put the subject in perspective, one has to realize that some of these complications are occurring in people who, had they not been treated in these ways, would, in fact, be dead and not available to have such complications. With that very brief introduction, we can go straight into the program.

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Complications of Intensive Megavoltage Radiotherapy for Hodgkin's Disease [1,2]

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SUMMARY—The complications observed in a series of 592 patients with Hodgkin's disease after treatment with intensive megavoltage radiotherapy have been reviewed. Radiation pneumonitis occurred in 6.4% of patients who received a single course of treatment to the mediastinum, in 15% of those reirradiated over the medastinum, and in 33% of those who received treatment to one or both lungs. Clinical severity and mortality were greater among the latter 2 groups. The introduction of the "thin" lung block shielding technique has sharply reduced the hazard of this complication. Radiation-induced heart disease developed in 32 patients (5.4%), of which 7 were severe, 4 requiring surgical intervention for chronic constrictive pericarditis, and 1 with a fatal outcome. The risk and severity of radiation-induced carditis rose sharply in patients who received $\geqq$2 courses of treatment over the heart (10/41, 24.4%). A modification of technique involving the insertion of a lead shielding block over the subcarinal area after a dose of 3000 rads appears to have virtually abolished this complication. Data on neurologic, gastrointestinal, and thyroid complications are also presented.—Natl Cancer Inst Monogr 36: 439–444, 1973.

THE INTRODUCTION of higher energies, permitting the use of higher doses and larger fields of treatment, has not only improved the long-term prognosis of patients with Hodgkin's disease, but, as might have been expected, also has yielded a significant number and spectrum of complications in a variety of normal tissues. Fortunately, only a few of these complications have been major, leading to severe, sometimes permanent disability or even death; most have been minor in severity and usually transient in duration. This paper briefly summarizes material which has been analyzed in greater detail elsewhere (*1*).

CASE MATERIAL

The data included in this analysis are based on a series of 592 biopsy-proved cases of Hodgkin's disease treated at Stanford University Medical Center between January 1964 and June 1970. Data on radiation-induced thyroid disturbances are derived from a separate analysis of a smaller series of patients studied by Glatstein et al. (*2*).

RESPIRATORY SYSTEM

The pathology and clinical features of radiation pneumonitis were described by Rubin and Casarett (*3*). In patients with Hodgkin's disease treated to the "mantle" field, there is usually

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[1] Presented at the International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Many of the studies included in this analysis were supported by Public Health Service research grant CA05838 from the National Cancer Institute.

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little or no clinical or roentgenographic evidence of pulmonary radiation reaction until about 3 months after the completion of treatment, when the mediastinal silhouette begins to appear rather fuzzy and shaggy, and irregular strands of density become evident in the paramediastinal pulmonary parenchyma in a distribution corresponding to the size and shape of the mediastinal portion of the mantle field. The patient during this period may have virtually no symptoms or may have developed a dry, hacking cough, sometimes accompanied by dyspnea on exertion or even at rest. Mild to moderate in severity, these symptoms usually persist for 2–4 months, then gradually diminish, disappear, and require no specific treatment.

When this mild form of pulmonary radiation reaction regresses, it does so permanently, leaving behind only minimal radiographic changes indicative of paramediastinal fibrosis, sometimes associated with elevation of the hilar vessels. The mediastinum may become slightly wider because of the paramediastinal fibrotic reaction fusing with the previous mediastinal silhouette.

In severe cases, such as are prone to occur when very large mediastinal masses must be irradiated, the onset may also be insidious, but the severity of symptomatology rapidly escalates, usually accompanied by the development of diffuse infiltrations of linear or mottled character in the affected segments or lobes of one or both lungs. Intractable cough, dyspnea, and air hunger, severe enough to require oxygen, may become evident and progress to cyanosis when both lungs are affected. Such patients are at serious risk of dying of acute pulmonary radiation reaction, and those who survive are likely to suffer a very prolonged convalescence, to manifest significant impairment of pulmonary ventilatory capacity for many years thereafter, and to be highly susceptible to recurrent bouts of pneumonia, further aggravating the fibrotic process.

As experience with the mantle technique has increased, refinements of technique have been introduced which have significantly diminished the hazard of severe pulmonary radiation reactions. In the "interruption" technique (*1*), a modest initial radiation dose is applied, and treatment is then interrupted to permit very large mediastinal masses to shrink, after which the program of radiotherapy is completed with the use of wider lung shields masking very tightly the reduced mediastinal silhouette. Another significant technical improvement has been the introduction of the "thin" lung block technique (*4*), which permits safe prophylactic irradiation of one or both lungs in cases with ipsilateral or bilateral hilar adenopathy. It delivers radiation to the lungs at a relatively low daily dose rate, so that a total dose of 1500 rads can be delivered to the lungs during the same 4-week period in which the mediastinum is receiving 4400 rads.

An analysis of the incidence of radiation pneumonitis in our patients is presented in table 1. Of 248 patients who received only a single course of treatment confined to the mediastinum, 16 (6.4%) subsequently developed radiation pneumonitis. Of these cases, 9 were mild to moderate and 7 were severe; 1 (0.25%) was fatal. However, the risk of radiation pneumonitis increased sharply when the mediastinum had to be reirradiated, particularly in patients referred to Stanford after inadequate radiotherapy elsewhere. In 6 of 41 patients (15%), radiation pneumonitis developed, which was severe in 5 and fatal in 1. However, the greatest risk of all occurred in patients in whom one or both lungs were treated, either therapeutically for pulmonary infiltrations due to Hodgkin's disease or prophylactically in the presence of significant hilar lymphadenopathy. Significant radiation pneumonitis developed in 23 (33%) of 69 patients in whom one or both lungs were treated, to a dose of approximately 1500 rads, by earlier techniques which employed high daily doses. Among these patients, 8 cases were severe, and 4 (5.8%) were fatal. More recently, with the introduction of the "thin" lead lung block technique (*4*), there have been no instances of symptomatic radiation pneumonitis associated with the delivery of the same total lung dose of 1500 rads at a lower daily dose rate over a period of approximately 4 weeks.

CARDIOVASCULAR SYSTEM

The heart has long been regarded as radioresistant, and it was therefore surprising when

TABLE 1.—*Incidence of radiation pneumonitis after one course of radiotherapy**

Time interval	Area treated	Number of patients at risk	Radiation pneumonitis	
			Number	Percent
Jan. 1964 to June 1969	Mediastinum only	248	16†	6
	Mediastinum and one or both lungs	69	23‡	33
Total		317	39	12
Jan. 1969 to June 1970	Mediastinum only	66	0	0
	Mediastinum and one or both lungs§	37	0	0
Total		103	0	0

*Stanford University Medical Center data, reproduced from Kaplan (*1*).

†One of these was fatal.

‡Four of these were fatal.

§Most of these cases were treated to a dose of 1500 rads in 4 weeks, with the thin lung block technique.

a number of patients with Hodgkin's disease who had been treated to a "mantle" field developed cardiomegaly, pericardial effusions, and other manifestations of radiation-induced heart disease. The clinical, pathologic, and radiologic features of these cases were fully described (*5–7*). Virtually all the documented cases were associated with total doses >3500 rads, delivered at the rate of 1000–1100 rads per week. The clinical manifestations included acute pericarditis, often associated with pericardial effusion and sometimes with signs of tamponade. Most of these manifestations spontaneously regressed with conservative medical management; e.g., the patient was frequently limited to bed rest and was given mild diuretics. However, a few of these patients progressed to chronic pericardial effusion and/or to constrictive pericarditis, sometimes requiring pericardiectomy. In severe cases, myocardial and/or endocardial fibrosis or fibroelastosis was observed. Most symptoms appeared within the 1st year after radiotherapy, but sometimes they were delayed as long as 4–5 years after radiotherapy. The clinical manifestations were those typical of pericarditis: fever, pleuritic chest pain, pericardial friction rubs, low voltage and other electrocardiographic abnormalities, enlargement of the cardiac silhouette on chest radiographs, and ultrasound or other evidence of pericardial effusion. The presence of neck-vein engorgement and of paradoxical pulse in some cases indicated the presence of tamponade.

In the 592 cases of Hodgkin's disease reviewed for this analysis, there was a total of 32 cases of radiation-induced heart disease (5.4%). Half of these were clinically asymptomatic and detected only radiographically. All have subsequently reverted to normal, either spontaneously or after the patient had had a short period of bed rest. In 9 other patients, symptomatology was mild to moderate and also reversible after bed rest and the use of diuretics. Only 7 cases were regarded as clinically severe, of which 4 progressed to chronic constrictive pericarditis requiring surgery, which had a fatal outcome in 1 patient (0.3%). Patients reirradiated to the mediastinum and cardiac silhouette because of recurrent Hodgkin's disease, after failure of a primary course of radiotherapy, were at much greater risk of developing severe radiation-induced heart disease. Of 41 patients who received ≧2 courses of treatment over the mediastinum, radiation-induced heart disease developed in 10 (24.4%), of which 4 were mild to moderate and 6 were severe, 4 of them (9.8%) being fatal.

Here again, refinements in technique have sharply alleviated the hazard of this potentially serious complication. Since its introduction about 3 years ago, the "subcarinal block" technique (*1*) has virtually abolished the hazard of radiation-induced heart disease. With this technique, the lower mediastinum and most of the midline cardiac volume are shielded when a dose of 3000 rads is delivered during treatment to the "mantle" field. The technique is feasible in patients with superior mediastinal, hilar, and even limited subcarinal lymphadenopathy, but cannot be used in the relatively infrequent cases in which lymphadenopathy is observed at the cardiophrenic angle or in the lower posterior mediastinum.

NERVOUS SYSTEM

The neurologic complications of Hodgkin's disease and of its treatment in this series of 592 patients are summarized in table 2. Goffinet et al. (*8*) recently presented additional data on herpes zoster. Their data were derived from a more complete analysis of patients who had undergone staging laparotomy with splenectomy, many of whom had also been treated with quadruple drug combination chemotherapy.

TABLE 2.—*Neurologic complications of Hodgkin's disease and its treatment**

	Number	Percent
1. Lhermitte's syndrome	62	11
2. Herpes zoster	91	18
3. Localized peripheral neuropathy	5	0.9
4. Radiation-induced transverse myelitis†	0	—
5. Severe motor disturbance (chronic Guillain-Barré syndrome)	3	0.5
6. Cerebellar dysfunction	1	0.15

*Stanford University Medical Center data on a series of 592 cases, reproduced from Kaplan (*1*).

†One case of presumed transverse myelitis previously recorded was subsequently shown at autopsy not to have had demonstrable lesions in the spinal cord.

There have been 2 cases of presumed transverse myelitis in our series, both of which were subsequently reclassified as presumptive instances of chronic Guillain-Barré syndrome. The first case, though closely simulating transverse myelitis, underwent virtually complete neurologic recovery after approximately 1 year. In the second case, the patient subsequently died of other causes and at autopsy no evidence was found of a neurologic lesion in the spinal cord. Occasional cases of transverse myelitis after intensive megavoltage radiotherapy for Hodgkin's disease have been reported from a number of other institutions. Apparently, in most of these cases, the gap left between the mantle and the para-aortic fields was insufficient and resulted in a small zone of high-dose overlap. Virtually all the instances observed to date probably could be traced to similar errors in technique. The hazard of transverse myelitis must be given particularly careful consideration in previously irradiated patients who develop new manifestations of disease requiring additional radiotherapy near the cervical or dorsal spinal cord.

The curious Lhermitte syndrome, described more fully elsewhere (*1*), is not a serious or major complication, since it has never been associated to date with motor dysfunction and has always spontaneously regressed completely, usually after 3–6 months. Whether this interesting neurologic disorder is etiologically related to Hodgkin's disease or its treatment is unknown.

LIVER

The syndrome of radiation hepatitis, fully described by Ingold et al. (*9*), is a dose-dependent complication, with an apparent threshold at a dose of 2500–3000 rads delivered at the rate of 1000 rads per week to the entire liver. It is apparently ascribable to selective injury to the central vein system of the liver. The recognition of this formerly unknown complication has led to modifications of technique, including the use of combined external radiotherapy and internally deposited radioactive gold colloid, which have diminished the hazard of radiation hepatitis and at the same time often effectively controlled Hodgkin's disease involving the liver (*10*).

GASTROINTESTINAL TRACT

Radiotherapy to lymph-node chains below the diaphragm, initially undertaken with a great deal of trepidation, has been remarkable for the paucity of associated significant gastrointestinal complications. Two patients had severe episodes of gastrointestinal bleeding, and two others had clinical and radiographic evidence of gastric ulcer. However, one other patient with a long-standing history of gastric ulcers was markedly relieved of his symptoms after radiotherapy, and one other patient had a massive gastrointestinal bleeding episode just before initiation of radiotherapy to the abdominal area. There has been one instance of chronic radiation enteritis, with stenosis, and one other instance in which radiation may have aggravated a postlaparotomy bowel obstruction with adhesions.

GENITOURINARY TRACT

Radiation nephritis is a well-established complication of radiotherapy when doses to the kidneys exceed 2500 rads (*11*). However, megavoltage radiotherapy beams make it possible to encompass para-aortic lymphadenopathy, as demonstrated on the lymphangiogram, with sharply collimated beams shaped to spare all of the right kidney and all but the upper pole of the left kidney. Accordingly, significant radiation nephritis has not developed in our patients with Hodgkin's disease treated with megavoltage radiotherapy. In the rare instances in which more massive para-aortic and/or mesenteric lymphadenopathy in the upper abdomen covers both kidneys, making it impossible to shield these organs through anteroposterior fields, the "interruption" technique and the "horizontal decubitis" technique (*1, 12*) may be used to deliver an adequate dose to the entire involved region, while shielding the kidneys adequately.

GONADS

The gonads are almost always injured when the pelvic field is irradiated. The aspermia observed in male patients is usually transient, particularly among younger individuals, several having become fathers of normal offspring within a few years after completion of radiotherapy. Only 3 documented instances of chronic aspermia or hypospermia which persisted long after completion of treatment of male patients with Hodgkin's disease were observed to date in our series, but no systematic effort was made to study sperm number or morphology at serial intervals after total lymphoid radiotherapy.

In female patients, ovarian function is usually permanently abolished when doses in excess of 500–800 rads are delivered to both ovaries. However, the introduction of oophoropexy, in which the ovaries are moved to the midline during the staging laparotomy, followed by the use of double-thickness lead blocks over the midportion of the pelvis to shield the displaced ovaries, has been helpful in circumventing this problem (*13*). At present, some 65–70% of the young female patients subjected to oophoropexy and subsequent pelvic radiotherapy have continued to exhibit or have later resumed normal menstrual function, and 2 patients proved fertile and were able to carry a normal pregnancy to term.

MISCELLANEOUS

Megavoltage radiotherapy spares the skin, and accordingly there are few significant complications involving the skin or subcutaneous tissues. Similarly, with the exception of the induction of hypothyroidism, few significant complications involve the endocrine system.

Clinical myxedema was observed in 5 of 124 (4%) of our patients with malignant lymphomas treated to the cervical region (*14*). A more recent study with various laboratory tests to detect more subtle degrees of hypothyroidism revealed significant hypofunction in 25 (11%) of 225 patients with malignant lymphomas (162 with Hodgkin's disease) who had both lymphangiography and subsequent radiotherapy to the head and neck region (*2*). Interestingly, 77 (44%) of 174 patients with malignant lymphomas thus treated developed elevated serum thyrotropin levels. Among these were 16 of the 19 patients (84%) who also developed radiation-induced pericarditis, which suggests that disturbance of thyroid function contributes to the hazard of this complication.

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SUMMARY OF INFORMAL DISCUSSION ON COMPLICATIONS OF RADIOTHERAPY

J. J. Nickson asked Doctor Kaplan to comment further about the problem of radiation hepatitis. **H. S. Kaplan** replied that it was a problem in the earlier cases in his series. He and his colleagues had published on all of those cases; some of them had gone on to a fatal termination. The word hepatitis is a misnomer; the pathophysiology is really that of hepatic veno-occlusive disease, and is virtually indistinguishable from Vaquez-Osler disease. It is associated with ascites, elevation of the serum alkaline phosphatase, and almost no problem with icterus. In nonfatal cases it seems to resolve and disappear, leaving a chronically scarred liver.

J. E. Ultmann asked whether radioisotopic scans of the liver had been obtained before and after treatment. **Kaplan** replied that scans routinely showed a failure of uptake by the left lobe of the liver after radiotherapy to the para-aortic node chains up to the diaphragm with a dose of 4000 rads in 4 weeks. However, the patients seem to get along very well with the two-thirds of the liver that they still have functioning. Recovery does take place slowly, and it seems to take over a year for patients to recover completely.

Question from audience: Are there any patients who exhibit a true idiosyncrasy to radiotherapy?

Kaplan said he presumed that the only answer to that question would be based on the occurrence of multiple complications in the same patient. So far as he could recall, only one patient had had a host of different complications, and that was a female patient with intestinal stenosis, radiation pericarditis, radiation pneumonitis, and hypothyroidism. But she also had one of the most massive mediastinal tumors that he had had occasion to treat, so the volume of her heart and lung that were subjected to very high dose was also very high. It is therefore difficult to know whether one is dealing with a true idiosyncrasy or with just greater risk due to large volume exposure in that particular patient.

Intensive Chemotherapy for Hodgkin's Disease: Long-Term Complications[1]

Vincent T. DeVita, *M.D.*, James C. Arseneau, *M.D.*, Richard J. Sherins, *M.D.*,[2] George P. Canellos, *M.D., and* Robert C. Young, *M.D., Medicine Branch, National Cancer Institute,*[3] *Bethesda, Maryland 20014*

SUMMARY—Long-term complications in patients treated with combination chemotherapy—nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP)—for Hodgkin's disease were assessed by evaluation of testicular function, immunocompetence, bone marrow function, and risk of development of second malignancies. No long-term impairment by chemotherapy of immunocompetence or bone marrow function was observed from our data, though more sophisticated measurements of the proliferative cell pools of these tissues may reveal some impairment not grossly apparent. Eight of ten patients treated with MOPP for 6 months were found to have been sterilized by chemotherapy. Testosterone and luteinizing hormone plasma levels were normal in all patients. Follicle-stimulating hormone levels were significantly elevated in sterile patients with absent germinal epithelium. Twelve second malignant tumors developing after therapy occurred in 425 patients with Hodgkin's disease treated with a variety of therapies, a 3.5-fold increase in risk over that calculated for a comparable normal population. Three second malignant tumors occurred in 35 patients treated with both intensive radiotherapy and intensive chemotherapy, a 29-fold increase in risk over that calculated for a comparable normal population.—Natl Cancer Inst Monogr 36: 447–454, 1973.

FOUR-DRUG-COMBINATION chemotherapy—nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP)—has been shown to be an effective therapy for previously untreated advanced Hodgkin's disease (*1*) and for Hodgkin's disease in relapse following radiotherapy (*2*). The acute toxicity of such therapy is predictable and is related to the effects of these cytotoxic drugs on cells with a rapid turnover and high rate of DNA synthesis. Limiting acute toxicity is produced by bone marrow suppression, with resultant hemorrhage and susceptibility to infection. In the hands of experienced therapists, such toxicity has not been of a magnitude sufficient to preclude therapy, and the improved median survival of patients so treated justifies the risk of these complications (*1, 2*).

Because of the recent introduction of this intensive chemotherapeutic program, there is little information about long-term toxicity. As with acute toxicity, chronic toxicity would be expected to be manifested mainly in organs with a high rate of cell turnover and DNA synthesis. Moreover, subjecting such tissues to repeated cytotoxic therapy over a long period might deplete the precursor cell pools responsible for recovery from more acute toxicity and/or alter the genetic material of these cells. These effects could give rise to such delayed complications as sterility in the male (the seminiferous tubules of the testes contain cells with a high turnover rate), chronic depression of bone marrow, impairment of immunocompetence, or predisposition to the development of second malignant tumors.

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[3] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

To evaluate the magnitude of such delayed complications, 57 previously untreated patients with Hodgkin's disease with at least 6 months of MOPP chemotherapy and no other further therapy were evaluated for delayed effects of chemotherapy on testicular function, immunocompetence, and bone marrow function. A group of 76 patients treated for at least 6 months with MOPP and 35 patients treated for at least 6 months with both MOPP and either total-nodal or extended-field radiotherapy (defined here as intensive radiotherapy) were evaluated for development of second malignant tumors.

METHODS

Testicular function.—Ten previously untreated patients with Hodgkin's disease were evaluated for testicular function from months to several years after completion of at least 6 months of MOPP chemotherapy (*3*). Each patient was evaluated with respect to karyotype, sperm counts, plasma testosterone levels, plasma gonadotropin levels, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (both baseline and in response to clomiphene stimulation), and testicular histology as assessed on biopsy specimens. All 10 patients had normal pubertal development and sexual potency; prior to developing Hodgkin's disease, 7 had fathered children; the other 3 were unmarried.

Immunocompetence.—Intradermal skin test data (delayed inflammatory response to purified protein derivative, coccidioidin, histoplasmin, and, in many cases, mumps and dinitrochlorobenzene challenge) were available before and after at least 6 months of MOPP chemotherapy in 13 previously untreated patients with Hodgkin's disease. A further 7 patients who had not been skin-tested before therapy received similar intradermal skin testing after completion of therapy.

Bone marrow function.—Hemoglobin, white blood cell (WBC) counts, differential count, and platelet counts were obtained from the charts of patients treated with MOPP for Hodgkin's disease for at least 6 months. Values were obtained for periods before therapy, at the conclusion of therapy, and during the unmaintained remission. Liver and renal function plasma chemistries were obtained from the charts of each patient for similar intervals.

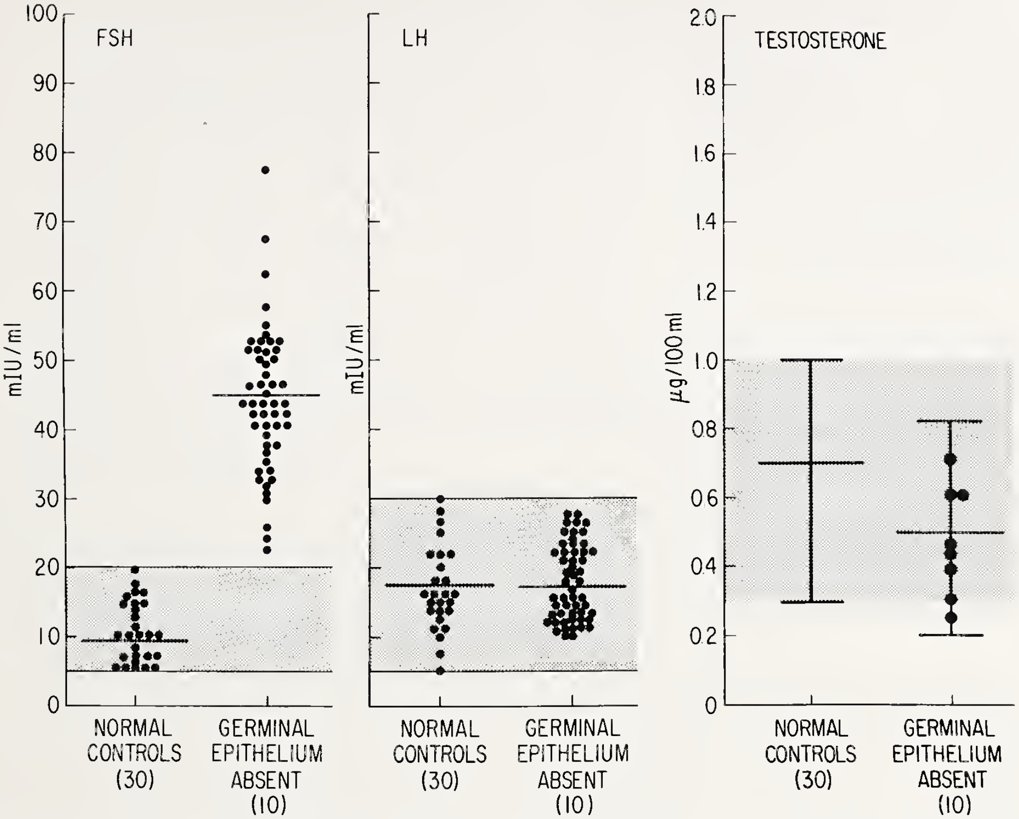
Development of second malignancies.—The charts of the 111 patients were surveyed for diagnoses of biopsy-proved malignancies other than Hodgkin's disease. These patients were a part of a larger review of second-malignancy development in 425 cases of Hodgkin's disease receiving either radiotherapy or chemotherapy or both (*4*). Age at diagnosis, sex, race, and period of observation from diagnosis to last follow-up, death, or development of a second malignant tumor were recorded for each case. Man-years of follow-up were calculated for each group of patients. With the use of cancer statistics (*5*), the number of tumors which would be expected in a normal population of equivalent age, sex, and race over such a period of observation was calculated. Confidence limits of 95% were then calculated to determine whether any increase of observed to expected tumors was statistically significant.

RESULTS

Testicular Function

All 10 patients had a normal male karyotype. Of the 10 patients, 8 showed either complete azospermia or very low sperm counts after repeated semen analyses; 6 of these had only Sertoli cells present within the seminiferous tubules on their testicular biopsies. One of the azospermic patients had only scattered spermatogonia and spermatocytes; the other patient had complete spermatogenesis in 30% of seminiferous tubules. Representative biopsy specimens comparing absent spermatogenesis with complete spermatogenesis are shown in figure 1.

All 10 patients had testosterone and LH levels within normal limits (text-fig. 1) and responded normally to clomiphene stimulation. FSH levels in patients with absent germinal epithelium were elevated significantly above both normal controls and postchemotherapy patients with intact germinal epithelium (text-fig. 1). FSH levels in all patients increased normally in response to clomiphene stimulation.



TEXT-FIGURE 1.—FSH, LH, and testosterone levels in postchemotherapy patients with absent spermatogenesis* and in normal controls.

*Includes data from 5 patients with lymphosarcoma also treated with prolonged chemotherapy.

Immunocompetence

Of the 13 patients skin-tested both before and after chemotherapy, 3 were anergic before and after chemotherapy, and 5 were reactive to at least 1 skin test before and after chemotherapy; 5 who were anergic before chemotherapy became reactive to at least 1 skin test after chemotherapy. The 7 who had not been tested before chemotherapy were all reactive to at least 1 skin test after chemotherapy. A patient reactive before chemotherapy never became anergic after chemotherapy.

Bone Marrow Function

Mean WBC and platelet counts were significantly decreased after 6 months of MOPP chemotherapy. Long-term follow-up, mean WBC and mean platelet counts had returned to the normal range (table 1). Mean hemoglobin and hematocrit values were significantly decreased in 7 of 57 previously untreated patients (17%). Since these patients required transfusions during chemotherapy, the mean hemoglobin following chemotherapy could not be calculated. Anemia caused by chemotherapy promptly recovered after cessation of therapy. Long-term follow-up, mean hemoglobin was within the normal range. Long-term follow-up, liver and renal function tests were within normal limits.

Development of Second Malignancies

Distribution of second malignant tumors among various therapeutic categories is shown in table 2. A significantly increased risk (3.5×) of development of second malignancies was observed in 425 patients. The most striking

TABLE 1.—*Mean blood counts before therapy, at the conclusion of therapy, and at yearly intervals after therapy of patients in remission who received no further treatment*

	Mean hemoglobin	Mean WBC count	Mean platelet count
Before therapy	Normal	7,800	234,000
At conclusion	*	3,300	182,000
12-month remission	13.5	6,900	250,000
24-month remission	13.9	7,300	245,000
36-month remission	14.2	6,300	230,000
48-month remission	12.9	6,100	205,000
60-month remission	14.5	7,000	200,000

*Not ascertainable because some patients required transfusion.

increase in risk was concentrated in the 35 patients treated with both MOPP and intensive radiotherapy (29×). The 76 patients treated with MOPP only did not show a significantly increased risk over that expected in a comparable normal population. A control group treated with intensive radiotherapy as the major therapeutic effort had a marginally increased risk (*4*).

TABLE 2.—*Evaluation of risk of development of second tumors in patients treated for Hodgkin's disease by various modalities*

			Second tumors		
	Number of cases	Follow-up (man-years)	Observed (O)	Expected (E)	O/E
All therapy	425	1584	12	3.383	3.547*
MOPP only	76	202	2	0.555	3.605
MOPP and total-nodal or extended-field radiotherapy	35	108	3	0.103	29.001*

*Statistically significant $P<0.05$.

The 3 second malignant tumors in the 35 patients treated with intensive radiotherapy and intensive chemotherapy all occurred in a group of 22 patients with Hodgkin's disease in relapse after initial treatment with intensive radiotherapy, who were treated with MOPP. These tumors, a squamous cell carcinoma of the skin, a fibrosarcoma, and a malignant mucin-producing tumor thought to be a mesothelioma, all developed in or near previously irradiated areas, displayed an unusually aggressive histology, and had a rapidly fatal clinical course. No second malignant tumors were observed in 13 patients who received MOPP before intensive radiotherapy. Because of the small numbers in these groups and the shorter follow-up in the 13 patients treated with MOPP before intensive radiotherapy (table 3), the difference in occurrence of second malignant tumors between these 2 groups may be due to chance.

DISCUSSION

Testicular Function

The seminiferous tubules of the testes contain a population of rapidly proliferating cells. It is expected that repeated exposure to intensive cytotoxic drug therapy would markedly affect such a tissue, and indeed, biopsies of 6 of 10 patients treated for at least 6 months with

TABLE 3.—*Age and duration of follow-up in 35 patients treated with intensive radiotherapy (IR) and intensive chemotherapy (IC)*

	Number	Mean age (yr)	Mean duration of follow-up (months from beginning of therapy)	Mean duration of follow-up (months from diagnosis of Hodgkin's disease)
Total	35	26.9	35.3	37.0
IR followed by IC	22	27.2	41.7	43.2
IC followed by IR	13	27.1	24.5	26.5

MOPP showed no spermatogenesis and 2 others had sperm counts below levels considered as the criterion for sterility. Therefore, 8 of 10 patients studied from months to years after completion of a course of intensive chemotherapy can be considered sterile. Toxicity of this type represents no physical threat to the patient. However, patients should be warned of the potential sterilization after therapy. Data on the effects of MOPP on ovarian function are not yet available.

The question of alteration of genetic material in patients not sterilized by intensive chemotherapy with the consequent risk of inheritable genetic defects is also of great concern. There is no information as to the magnitude of this risk at present.

Immunocompetence

While patients are in the process of treatment with MOPP, they are immunosuppressed for at least some of the time because of the direct and indirect effects of the drugs on immunocompetent cells. However, results of skin tests of delayed hypersensitivity in 20 patients treated with MOPP indicate an actual improvement in the status of the cellular immune mechanisms (as assessed by these tests) after chemotherapy in some patients. This finding, and the fact that no patient skin-test reactive before chemotherapy was anergic after chemotherapy, suggest that anergy in Hodgkin's disease is associated with activity of the disease and quantity of disease present. This conclusion is supported by the recent findings of Young et al. (*6*).

The recovery of skin-test reactivity after chemotherapy in some patients probably reflects the response of the Hodgkin's disease to chemotherapy. We conclude that MOPP chemotherapy has no long-term detrimental effect on immunologic competence as assessed by skin tests of delayed hypersensitivity and may, in fact, improve an anergic patient's ability to respond to intradermal skin tests, provided his Hodgkin's disease responds to therapy.

Bone Marrow Function

No long-term suppression of peripheral WBC count, platelet count, or hemoglobin was found in patients receiving MOPP for 6 months. This observation suggests that bone marrow function following such chemotherapy is adequate to maintain normal peripheral counts, at least in an unstressed situation. The measurement of peripheral blood counts, however, is a relatively crude measurement of the proliferative cell pool of the bone marrow. As previously mentioned, these cells would be expected to be most sensitive to the cytotoxic agents employed in the treatment. Studies directed at more specific measurement of the bone marrow's proliferative capacity, such as bone marrow colony-forming capacity, are currently in progress.

Development of Second Malignancies

Increased susceptibility to development of second malignancies in Hodgkin's disease has been the subject of previous reports (*7–10*). Most authors have concluded that aside from an increased incidence of skin cancers and Kaposi's sarcoma, the risk of development of second tumors in patients with Hodgkin's disease is not greater than that of a normal control population (*9, 10*). Crosby, however, estimated that the incidence of acute granulocytic leukemia in patients with Hodgkin's disease is 10 times that of the general population (*11*). He suggested that this increased risk may be related to radiotherapy received by these patients.

We have found a 3.5-fold increase in the risk of development of second malignant tumors in 425 patients with Hodgkin's disease treated with a variety of therapies and a 29-fold increase in the risk of development of second malignancies in 35 patients treated with both intensive radiotherapy and intensive chemotherapy. The high risk observed in the latter group raises questions regarding possible oncogenic factors within this group.

The immunologic abnormalities associated with Hodgkin's disease have been well studied (*12*). Other diseases with evidence of immunodeficiency at some time in their course, such as chronic lymphocytic leukemia and various congenital immunodeficiency diseases, have been more definitely associated with an increased risk of development of malignant tumors than has Hodgkin's disease (*13, 14*). All 35 patients within the highest risk therapeutic subgroup received for prolonged periods large doses of both immunosuppressive radiotherapy and immunosuppressive chemotherapy. Studies of chronic immunosuppressive therapy in renal transplant recipients reveal an increased risk of developing malignant tumors (*15*). The combination of immunologic abnormalities associated with the continuously active Hodgkin's disease of the 22 patients in whom the second malignant tumors were observed (all of whose disease had relapsed following radiotherapy) and the iatrogenic immunosuppression of the radiotherapy and chemotherapy to which they were exposed, may have been important in causing the high incidence of development of second malignancy. Such importance is, however, difficult to assess in retrospect.

In addition to their immunosuppressive effects, both radiotherapy and cytotoxic chemotherapy have been extensively associated with oncogenesis in animals and humans (*16–20*). The identification of a high-risk subgroup treated with both intensive radiotherapy and intensive chemotherapy suggests that the cumulative cellular damage induced by the combined therapy and/or the possible impairment of cellular repair mechanisms (which may be induced by one mode of therapy on the cellular lesions produced by the other mode of therapy, either through further immunosuppression or further additive damage to cellular DNA) may be an important factor in accounting for the increased incidence of second malignant tumors occurring in patients so treated.

Patients with relapse after intensive radiotherapy have reacted to intensive chemotherapy in a fashion similar to previously untreated patients, and also have comparable remission rates and duration of remission following chemotherapy (*2*). When the therapeutic gain is balanced against the risk of developing a second malignant tumor in these patients, chemotherapy is easily justified because of their poor prognosis. The use of a combined approach in patients with localized disease, but with a high risk of recurrence, may be hazardous and should be balanced against the risk of the development of a second malignancy. One other factor should be considered. Patients who receive either chemotherapy or radiotherapy after relapse have an additional factor which may play a role in the development of a second malignancy, the continued presence and antigenic stimulation provided by the growing tumor tissue. The clinical setting is analogous to that of renal transplantation where the donated kidney provides a source of continued antigenic stimulation to the host while the host's immunologic system is suppressed by drugs, a circumstance associated with an increased incidence of malignancies (*15*).

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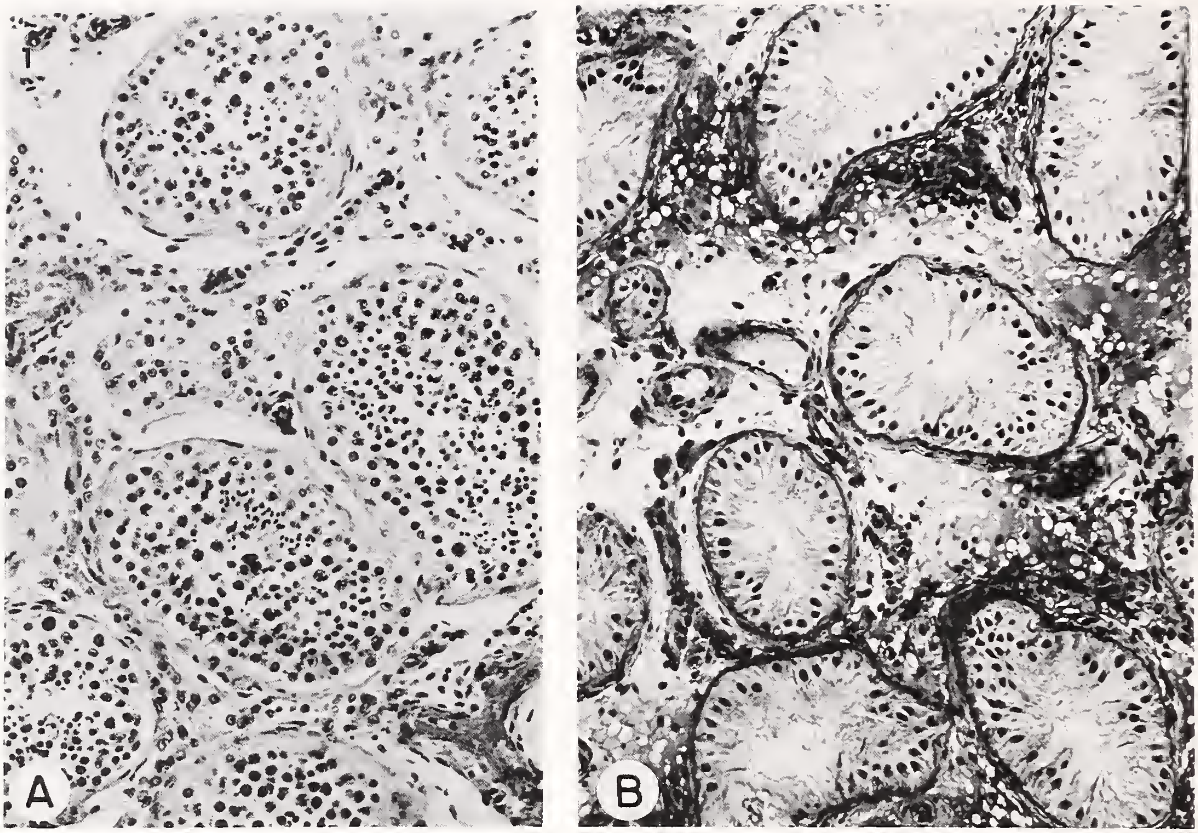

FIGURE 1.—Representative testicular biopsy sections. *A*: Normal biopsy showing complete spermatogenesis. *B*: Biopsy of a postchemotherapy patient showing absent spermatogenesis. Only Sertoli cells are present within seminiferous tubules.

SUMMARY OF INFORMAL DISCUSSION ON COMPLICATIONS OF CHEMOTHERAPY

S. A. Rosenberg commented that Doctor DeVita's observations about second tumors are very provocative. However, he wondered about the statistics involved. It is certainly very striking to see such a high incidence in one subgroup but the way it has been achieved has been to identify the variables and then to narrow down to a group of 7 patients. The figures cannot be ignored, but can one say they are statistically significant? **V. T. DeVita** replied that they are statistically significant when analyzed as two-by-two tables. Whether they are biologically significant is another question. The group is very small, but it bears mentioning. We will have to wait until a larger number of patients treated in this fashion are at risk for a long enough interval. There is one factor in this group of patients that is different and it may be an important difference, i.e., that these patients have had evidence of active Hodgkin's disease when they were started on chemotherapy. They are different than a group of patients who receive radiotherapy and are in complete remission when given chemotherapy. That may seem to be a trivial point, but some people believe that the high incidence of tumors in the immunosuppressed renal transplant patients is related to the constant antigenic stimulus by the transplanted kidneys so they may be a different population.

E. Frei commented that, of all the chemotherapeutic agents employed in Hodgkin's disease, procarbazine is the only one known to be carcinogenic in experimental animals. Was the chemotherapy in Doctor DeVita's cases always MOPP (mechlorethamine, vincristine, procarbazine, and prednisone combined)? **DeVita** said that it had been MOPP in all of the patients with second neoplasms. **Frei** asked whether there were any data on a control group that excluded procarbazine. **DeVita** answered that there were no such data. All of these patients had received MOPP. The only evidence that he could cite suggesting an effect of both therapies is that the 110 patients with fairly long follow-up periods who were treated with MOPP alone, including procarbazine, had had no unusual increase of second tumors. These tumors appeared at 1, 2½, and 5 years from the end of all therapy, so the time span may be a bit longer than the group followed at Stanford University, who received both types of therapy.

H. S. Kaplan asked Doctor DeVita to comment regarding teratogenic effects. The question one frequently hears is not about the hazard of sterilizing the female, but that of producing an abnormal child. Did Doctor DeVita or anyone else in the audience have any data on this point, either after radiotherapy or chemotherapy? **DeVita** answered that in the first 43 patients there have been 5 pregnancies. All have terminated normally, and the children appear perfectly normal. He did not know of any instance of an abnormal pregnancy. It seems that if the patients can conceive, their children will probably be all right. However, definitive information on that point would require a megapatient study; it is really difficult to quantitate that kind of data in a small group.

M. Tubiana asked whether Doctor DeVita's group had performed experiments in animals to study the carcinogenic effects of the association of chemotherapy and radiotherapy. **DeVita** said they had not, but, as Doctor Frei pointed out, procarbazine alone is probably the most potent carcinogen now in use in the clinics.

Complications From Staging Laparotomy for Hodgkin's Disease [1,2]

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SUMMARY—Staging laparotomy consisting of splenectomy, liver, lymph-node, and bone-marrow biopsies was performed in 291 patients with Hodgkin's disease as part of their initial diagnostic workup over the past 3½ years. The operative mortality rate was zero. The morbidity rate was 12% with all but one of the complications managed successfully with appropriate therapy.—Natl Cancer Inst Monogr 36: 457–459, 1973.

EXPLORATORY LAPAROTOMY including splenectomy, liver biopsy, and para-aortic lymph-node biopsy has now become an integral part of the diagnostic workup for patients with Hodgkin's disease (*1*). Accurate staging is essential, since the effectiveness of radiotherapy depends on the recognition of all disease-bearing sites. The staging laparotomy has resulted in a change in the clinicopathologic stage in up to 50% of cases (*2*). Improved cure rates as a result are anticipated. An earlier report (*2*) from this Institution presented the operative morbidity statistics for the initial group of patients undergoing this surgical procedure. Since then, our series has rapidly enlarged. This article reviews the postoperative complications from 291 consecutive staging laparotomies performed between July 1, 1968, and March 1, 1972.

PATIENT GROUP AND TECHNIQUE

All patients underwent staging laparotomy as part of their *initial* diagnostic workup at Stanford University Medical Center. None had received prior radiotherapy or chemotherapy. Not included in this report are a number of patients who underwent exploration to evaluate possible recurrent abdominal disease after having already received a course of therapy. The median age was 25 years; the mean age was 28 years. The age range was 18 months to 65 years (table 1). There were more males than females in the group.

TABLE 1.—*Patient population—staging laparotomy for Hodgkin's disease, Stanford University Medical Center*

291 Operations—July 1, 1968–March 1, 1972

Mean age 28 years; median age 25 years

179 males; 112 females

Age range: 18 months–65 years

Decade	1	2	3	4	5	6	7
Number of cases	11	64	110	56	21	19	8

The operative procedure, previously described (*2*), consisted of splenectomy, liver biopsy (both wedge and Vim-Silverman needle), biopsy of any suspicious node on lymphangiogram or upper-left para-aortic lymph-node biopsy, if the lymphangiogram was negative, and open iliac crest bone-marrow biopsy. The celiac, porta hepatic,

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[2] The staging laparotomies and other diagnostic procedures on these patients were supported in part by Public Health Service grants CA05838 and CA08122 from the National Cancer Institute.

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and mesenteric nodes were thoroughly examined by palpation but not routinely biopsied.

We performed the splenectomy by mobilizing the spleen into the operative field and ligating the splenic vessels close to the pancreas to allow a wide sampling of the splenic hilar lymph nodes. A large tantalum clip was then placed on the splenic vascular pedicle to act as a marker for the radiotherapist in designing the fields for treatment. Smaller tantalum clips were used at the para-aortic node biopsy site for hemostasis and to aid the radiotherapist in assessing which nodes were removed. (The clips were also placed on any other grossly abnormal nodes situated outside of the normal "inverted-Y" radiotherapy field.) The bone-marrow biopsy was routinely performed on the left iliac crest with the use of a Stryker saw mechanism adapted to remove a 1-inch core of bone and marrow. In addition, in 48 patients oophoropexy was performed—moving the ovaries to a midline position where they can be protected by lead shields from the anticipated radiotherapy (*3*). This procedure consists of sewing one or both of the ovaries to the posterior wall of the uterus, as low as possible, with a single wire suture.

A number of ancillary procedures were also performed, including incidental appendectomy (4 cases), oophorectomy for tumor (2 cases), cholecystectomy (2 cases), colectomy for tumor, Meckel's diverticulectomy, and tubal ligation (2 cases).

RESULTS

There were no operative deaths. All the patients were discharged from the hospital after staging laparotomy; the average postoperative stay was 7 days. One patient died 4 months after operation from complications probably in part related to his surgery. This patient was a 34-year-old male with Stage IV-B Hodgkin's disease with pelvic bone involvement and daily preoperative temperatures to 40° C. His staging laparotomy and initial postoperative course were unremarkable except for the fever. Six weeks postoperatively he presented in shock at another hospital; re-exploration revealed intra-abdominal bleeding from a subcapsular hepatic hematoma and oozing from the splenic pedicle. His condition stabilized for 2 months thereafter, but he subsequently died from sepsis and disseminated Hodgkin's disease aften an intensive course of chemotherapy.

The overall complication rate was 11.7%. There were 8 major complications for a rate of 4% (table 2). All of these problems were satisfactorily managed with appropriate medical treatment. Two patients developed pneumonia which responded to vigorous pulmonary toilet and antibiotics. A 19-year-old male developed a pulmonary embolus from a severe ileo-femoral thrombosis 12 days post operation. He was managed with intravenous heparin for 3 weeks with resolution of all signs of phlebitis thereafter. He was continued on oral anticoagulation for an additional 3 months.

TABLE 2.—*Major complications—staging laparotomy for Hodgkin's disease: 291 patients*

Major complications	Number of cases	Percent
Pneumonia	2	<1
Pulmonary embolus	1	<1
Fecal fistula	1	<1
Small-bowel obstruction	2	<1
Wound dehiscence	1	<1
Ureteral transection	1	<1
Total major complications	8	3.7

One patient, a 40-year-old male, developed a partial small-bowel obstruction 3 weeks post operation and was treated by lysis of an adhesion to the underside of his midline incision, after lack of response to conservative management with a Cantor tube. Another patient, a 62-year-old male, developed a bowel obstruction 6 months post operation. He had completed a full course of abdominal radiotherapy and presented with an "acute abdomen." Laparotomy revealed 2 feet of gangrenous small bowel in a closed-loop type of obstruction thought to be due to adhesions. The postoperative course of both of these patients was unremarkable.

A fecal fistula resulted from an intraoperative laceration of the splenic flexure of the colon during splenectomy. The laceration was identified and closed, and the left subphrenic space was drained. Purulent, and subsequently fecal, drainage developed from the drain site, but this fistula closed spontaneously by the 4th postoperative week.

One patient sustained a traumatic laceration of his left ureter during para-aortic node dissection. The ureter was re-anastomosed without a stent, and the postoperative intravenous pyelogram was entirely normal.

There were 34 minor complications—rate of 9% (table 3). Wound infections developed in 11 patients (4%) and were managed by open drainage, frequent wound irrigations, and appropriate systemic antibiotics when indicated.

TABLE 3.—*Minor complications—staging laparotomy for Hodgkin's disease*

Minor complications	Number of cases	Percent
Wound infection	11	4
Abdominal	8	3
Iliac crest	3	1
Urinary tract infection	4	1
Pancreatitis (mild)	1	1
Subphrenic fluid accumulation	1	1
Prolonged ileus	2	1
Atelectasis	4	1
Division lateral femoral cutaneous nerve	3	1
Total minor complications	26	9

There have been no complications from the oophoropexy procedure, and 2 patients in this group have delivered normal babies after having received abdominal and pelvic radiotherapy.

Eleven patients, 4% of the group, received whole-blood transfusions either during the operation or during the postoperative period as replacement for operative blood loss. One patient received 3 U, 6 patients received 2 U, and 4 patients received 1 U.

DISCUSSION

Staging laparotomy is indicated only if the benefit from the information to be obtained exceeds the risk of the operative procedure. Certainly, the mortality rate should approach zero because the patient population is relatively young and the operation is done electively and under optimal conditions. The morbidity rate should also be low, though a small percentage of patients will develop unavoidable complications. In our series, the ureteral transection, colon fistula, and lateral femoral cutaneous nerve injuries were obviously a result of technical errors and are avoidable. The latter nerve injuries, for example, occurred during performance of the bone-marrow biopsy at the wrong point on the iliac crest in 3 successive patients.

We do not routinely drain the left subphrenic space post splenectomy. In fact, the only subphrenic fluid accumulation developed in a patient in whom the surgeon thought that the tail of the pancreas had been injured and a penrose drain was left. This patient subsequently drained purulent material from the drain site, but this draining ceased spontaneously and reoperation was not required. The surgical literature seems to support this point of view (*4*, *5*). Again, to keep the complication rate at a minimum, we do not perform incidental appendectomy.

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SUMMARY OF INFORMAL DISCUSSION ON COMPLICATIONS OF STAGING LAPAROTOMY

C. Berard commented that a number of patients are now being referred to the National Cancer Institute who have had laparotomies at the referring institution, and often these have been inadequate laparotomies. He asked whether Doctor Nelsen re-explored patients at Stanford University Medical Center who have had incomplete laparotomies elsewhere. **T. S. Nelsen** replied that he did so with great reluctance. It is actually not so hard to do if the spleen has been removed. It is very hard on the patient, but not technically difficult as a rule.

S. Hellman asked about the mean ages of the 2 groups of lymphoma patients. He was struck by the difference in complication rates and wondered if this could be related to age differences. **D. R. Goffinet** responded that the mean age of the laparotomy patients with non-Hodgkin's lymphomas was 44–45 years (approximately). **Nelsen** added that the mean age of the Hodgkin's disease group was 28 years, so there is a great difference in age and also in their general condition, of course. **S. A. Rosenberg** commented that Doctors Donaldson, Moore, and Vosti have looked at the sepsis that occurred post splenectomy in this large group of lymphoma patients, comparing it to other patients who have had splenectomy at Stanford University Medical Center. There was no significant increase in overall risk of sepsis. However, there were 5 cases of pneumococcus sepsis in the Hodgkin's disease postsplenectomy group. There were also 2 deaths in the splenectomized Hodgkin's disease patients, one from *Hemophilus influenzae* in a 9 year old and the other from bilateral pneumococcal pneumonia in an 11 year old. Both of these patients also had been treated with intensive chemotherapy and radiotherapy, but they had the type of demise which has been associated with splenectomy and they were young people. He felt that splenectomy has to be at least considered as a potential contributing factor in such a case.

G. H. Fairley added that one of the very early deaths with combination chemotherapy in the British series was one just as Doctor Rosenberg described. This patient was a man who came in actually with 3 simultaneous infections: the Guillain-Barré syndrome, generalized herpes zoster, and pneumonia. Now if somebody had removed his spleen, this might have been considered contributory, but he had not had a splenectomy. We must be very careful not to attribute such instances to the absence of a spleen when we have also given combination chemotherapy, particularly combinations which include steroids and perhaps also radiotherapy.

Herpes Zoster Infections in Lymphoma Patients[1,2]

Don R. Goffinet, ***M.D.,*** **Eli Glatstein,** ***M.D., and*** **Henry S. Kaplan,** ***M.D., The Paul A. Bissinger Radiotherapy Center, Department of Radiology, Stanford University Medical Center, Stanford, California 94305***

SUMMARY—The increased incidence of herpes zoster infections found in the most recently treated patients with Hodgkin's disease at the Stanford University Medical Center was not seen in those with non-Hodgkin's lymphomas. This increase in number of infections occurred after combination chemotherapy and routine staging laparotomy with splenectomy were instituted, and several years after extended-field or total-nodal versus local-field radiotherapy as randomized therapeutic options had begun. It is probable that splenectomy played a role in the eventual development of herpes zoster infections in patients with Hodgkin's disease, when combined with extended-field radiation therapy and/or chemotherapy.— Natl Cancer Inst Monogr 36: 463–464, 1973.

THE RECORDS of 1,130 patients with Hodgkin's disease and other lymphomas seen in both the Stanford University Lymphoma and Radiation Therapy Clinics between 1959 and 1970 were reviewed, as previously described (*1*).

The tabulated data revealed an increased number and percentage of herpes zoster-varicella infections in 1969, the year most recently examined. This increased number of infections could be explained neither by more patients at risk nor by a clustering of cases, since there was no seasonal change in numbers of herpes zoster infections and 85% of the cases occurred within 1 year of the initiation of chemotherapy and/or radiation therapy.

Extended-field (EF) radiation therapy was used increasingly at Stanford throughout the early 1960's, along with local-field (LF) irradiation, but the routine use of total-nodal (TN) radiotherapy (versus LF treatment as a part of a treatment protocol) was not begun until 1966–67 for patients with Hodgkin's disease. The number of cases of herpes zoster infection, however, did not increase sharply until about 2 years after this change in treatment policy and coincided with the initiation of MOPP (a combination of mechlorethamine, vincristine, procarbazine, and prednisone) chemotherapy and routine staging laparotomy with splenectomy for patients with Hodgkin's disease (table 1).

We found a significantly increased incidence of herpes zoster-varicella infections in those patients with Hodgkin's disease who underwent splenectomy as compared to those who did not (33/150 patients vs. 58/442; $\chi^2=6.1$, $P<0.05$ but >0.01). The difference between the two groups was still significant even when the patients who had received MOPP chemotherapy were removed from consideration. Though the numbers of cases at risk were too small to be significant, MOPP combination chemotherapy also seemed to be associated with an increased incidence of herpes zoster infection. That the extent of the radiotherapy fields was probably also a factor is indicated by the fact that, whereas LF radiotherapy was employed in 151 of 482 cases (31%), it accounted for only 18

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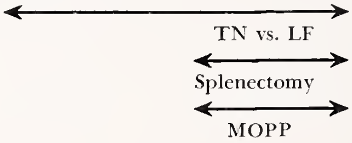
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported in part by Public Health Service grants CA05838 and CA08122 from the National Cancer Institute.

TABLE 1.—*Hodgkin's disease*

	Year											
	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	Total
Number of herpes zoster infections	0	2	2	1	4	4	8	10	10	21	29	91
Number of new cases of Hodgkin's disease seen each year	19	13	18	24	33	59	57	73	92	114	90	592
Treatment fields												
LF	5	1	6	12	10	13	13	19	22	30	20	151
EF or TN	2	2	6	9	18	27	35	45	59	66	62	331



(20%) of the 89 cases of herpes zoster in which radiotherapy had been administered; EF or TN treatment had been given to the remainder ($\chi^2=5.6$, $0.01<P>0.05$).

The clinical course of patients with these infections in our splenectomized group was not apparently worsened by this procedure, nor did patients exhibit a greater tendency to dissemination of their infections.

Therefore, although it is difficult to separate the effects of recent changes in treatment and pretherapy evaluation policies on the eventual development of herpes zoster infections, we believe that splenectomy plays a role in this process, when combined with EF radiation therapy and/or chemotherapy.

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Herpes Zoster: Recent Studies [1,2]

David A. Stevens, *M.D., and* **Thomas C. Merigan,** *M.D., Division of Infectious Diseases, Department of Medicine, Stanford University Medical Center, Stanford, California 94305*

SUMMARY—Dissemination of herpes zoster has been studied with regard to host immune factors. The kinetics of vesicle fluid interferon appearance correlates most closely with the course of the disease. Cessation of dissemination follows development of peak vesicle interferon levels. Local interferon may be an important defense against viral infections, and local production may be related to cell-mediated immunity.—Natl Cancer Inst Monogr 36: 465–468, 1973.

HERPES ZOSTER usually appears as a localized infection confined unilaterally to a dermatome served by the nerve distribution of a spinal cord segment or cranial nerve branch. However, sometimes the infection spreads from its localized area and pox appear in distant areas of the skin; visceral involvement may also occur. We report here our studies of the influence on dissemination of several host immune factors, including humoral immunity, cell-mediated immunity, and interferon.

Between July 1969 and April 1971, 151 patients were referred to our group and were the most intensively studied. Of these patients, 42 had disseminated disease. The patients were divided into 3 categories: no underlying disease, or "normal," concurrent Hodgkin's disease, and miscellaneous. Our analyses of the effects of diagnosis, stage of disease, and type of therapy were detailed elsewhere (*1*).

Circulating lymphocyte counts, delayed hypersensitivity to 6 skin test antigens, lymphocyte stimulation by phytohemagglutinin, and quantitative immunoglobulins were compared for localized and disseminated zoster in the 3 patient categories (table 1). Although all these parameters were more commonly depressed in the patients with associated diseases, there was no correlation with dissemination within any of the patient categories.

Table 2 illustrates the results of complement-fixing antibody determinations in the first 15 days after onset in 125 patients with localized and disseminated disease. For each 5-day interval after onset, more patients with localized disease had antibody. The mean titer and peak titer eventually reached by both localized and disseminated patients were the same. The relative unimportance of antibody alone in preventing dissemination is suggested by the finding that 5 patients began to form antibody before dissemination began. This group included a patient with a titer of 1:128 four days before dissemination began. Thus, presence of complement-fixing antibody alone does not reliably predict whether dissemination will occur.

The kinetics of vesicle fluid interferon (V-IF) appearance was studied with serial specimens. We found both patients with localized and disseminated disease began with low levels—<1000 U and commonly <100 U—which then later rose, usually abruptly over a 1- to 2-day period, to a peak value. These levels declined as pustulation and then crusting occurred. However,

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported by Public Health Service grant AI05629 from the National Institute of Allergy and Infectious Diseases.

TABLE 1.—*Correlation of impairment of host immunity and dissemination**

Immune deficiency	Patient category†		
	Normal	Miscellaneous	Hodgkin's disease
Lymphopenia in:			
Localized zoster	0/19	15/27	5/7
Disseminated zoster	1/9	5/13	12/17
Anergy in:			
Localized zoster	7/12	8/10	4/4
Disseminated zoster	0/4	3/4	11/12
Depressed IgG, IgA, or IgM in:			
Localized zoster	4/35	2/18	3/5
Disseminated zoster	1/7	4/7	3/9
Depressed lymphocyte transformation to phytohemagglutinin in:			
Localized zoster	1/15	7/16	3/5
Disseminated zoster	0/6	2/5	6/12

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†Fractions are number of patients in category with immune deficiency/total patients in category assayed for this immune parameter.

TABLE 2.—*Percent of patients with complement-fixing antibody at selected time intervals**

	Time interval (days after onset)†		
	0–5	6–10	11–15
Localized zoster	50% (26)	79% (47)	98% (64)
Disseminated zoster	26% (19)	55% (22)	77% (26)

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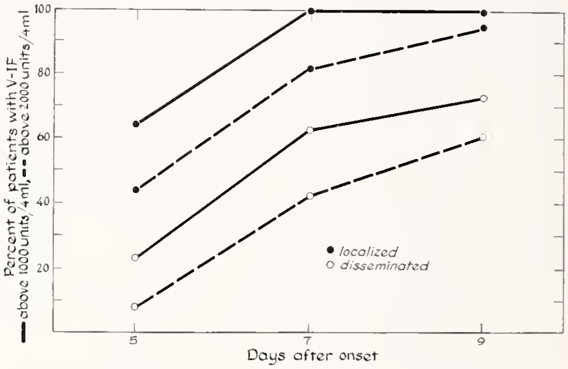
†Percent = No. of patients with antibody titer >1:8/total patients (No. in parentheses) for whom data were available × 100.



TEXT-FIGURE 1.—Differences in V-IF appearance in 25 localized and 22 disseminated cases of zoster as shown by time of development of intermediate (1000–2000 U/4 ml) V-IF levels. Differences between localized and disseminated zoster at both levels at 5, 7, and 9 days are significant at $P < 0.05$.

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patients with localized disease had an earlier rise in V-IF levels. At 5 days after onset of disease, most patients with localized disease had reached intermediate levels such as 1000 U, and at 9 days all had done so. These patients were significantly different ($P<0.05$) than the patients with disseminated disease at this level at 5, 7, *or* 9 days after onset (text-fig. 1).

Sufficient serial samples were available from 43 patients (April 1969 to March 1972) to determine on which day the sharp rise in V-IF began, and this information demonstrated *also* the earlier rise of V-IF in patients with localized disease (table 3). All 13 patients with localized disease had begun their sharpest increase within 8 days after onset, whereas only 17 of 30 patients with disseminated disease had done so. The extent of the increase was the same in patients with localized and disseminated disease. The mean increase in V-IF was approximately 10,000 U in both groups. The observed differences between localized and disseminated patients in interferon kinetics appeared independent of any of our 3 patient categories.

TABLE 3.—*V-IF response*

Days from onset to maximum rise	Localized zoster	Disseminated zoster
Mean	5.3	9.4
Range	1–8	2–32
≤8 days	13/13	17/30

The cessation of dissemination closely correlated with the development of peak V-IF levels (table 4). In 21 of 21 patients with adequate serial specimens and close follow-up with circling and counting of lesions, dissemination ceased within 48 hours after peak V-IF titers were reached, regardless of the number of days after onset or the number of days of dissemination which had elapsed. In 9 of 25 patients, complement-fixing antibody was clearly absent during this period and did not appear until *after* dissemination had ceased. These analyses exclude patients who received concurrent cytosine arabinoside therapy (*2*).

TABLE 4.—*Events within 48 hours of cessation of dissemination*

	Yes	No
Peak V-IF titer	21	0
Presence of complement-fixing antibody	16	9

We also noted that, although the vesicles had contained clear fluid until that time, within 24–48 hours after dissemination ceased, clouding of the vesicle fluid peripherally and in the primary dermatome occurred, i.e., pustulation, and drying and crusting began shortly thereafter.

We found that patients with localized and disseminated zoster, if studied serially, began with low V-IF levels which then rose to the same extent to high levels in both groups. A few patients with intermediate levels, i.e., about 1000 U but <2000 U, also had dissemination which, however, ceased as V-IF levels continued to rise. No patients with localized disease and a V-IF level of >2000 U have been observed to have dissemination. When a patient with disseminating disease reaches this level, dissemination ceases within 48 hours.

We can only speculate that high V-IF titers are causally related to cessation of dissemination, but the timing of these 2 events is appropriate for such a hypothesis. A local interferon response, perhaps mediated by sensitized lymphocytes migrating into the skin, may be critical in halting virus replication and thus preventing viremia. That sensitized lymphocytes release interferon in response to nonviral antigens has been shown in vitro, and it has been demonstrated that lymphocytes may be the critical effector cell in interferon production in some animal models of virus infection. Cessation may of course occur as a result of change in another variable, not sampled, which parallels V-IF development, or that acts in conjunction with it. Such a variable could be a type of antibody other than complement-fixing antibody—e.g., neutralizing antibody—or a contribution of the cellular immune system.

We hypothesize that, if an individual can mobilize a prompt systemic anamnestic humoral response, perhaps demonstrable by neutralizing antibody levels, as well as a local response in the primary dermatome mediated by sensitized lymphocytes and their effector molecules, he will contain the infection despite any associated disease or immunosuppressive therapy. This interpretation of our findings indicates further immunologic studies that will be needed, and has implications for therapeutic intervention in this disease. Our data also suggest a potentially important role for local interferon in host defense against viral infections.

We are currently exploring the possible role of humoral immunity in preventing dissemination via passive antibody transfer. We are comparing the effect of pooled normal γ-globulin versus immune globulin prepared by plasmaphoresis from the plasma of patients convalescent from zoster who have high antibody titers —zoster-immune globulin, or "ZIG." These reagents are being compared in compromised hosts with localized zoster who have a high risk of dissemination, i.e., patients with a malignancy or who are receiving cytotoxic drugs.

Cytosine arabinoside, an inhibitor of pyrimidine synthesis, has been shown to inhibit DNA virus replication in vitro and has been claimed to be effective in the treatment of virus infections of man. We are currently also evaluating, in double-blind controlled fashion, the effect of this reagent on cases of disseminated zoster.

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SUMMARY OF INFORMAL DISCUSSION ON INFECTIOUS COMPLICATIONS

S. A. Rosenberg said he had expressed disbelief in the figures Doctor Stevens presented 2 years ago that most of the Stanford University patients with Hodgkin's disease, when followed prospectively after the onset of herpes zoster, went on to disseminate. He asked if Doctor Stevens could give the current figures on the frequency of dissemination of Hodgkin's disease? **D. A. Stevens** replied that, at the time of the prior analysis period (July 1969 to April 1971), there were 26 patients with Hodgkin's disease and herpes zoster, and the dissemination rate was 73% in that group of patients. In the following year, there had been approximately an equal number of patients and the dissemination rate was still $\geqq 50\%$. **P. P. Carbone** commented that the Stanford University incidence of zoster is extremely high. It reminded him of the National Cancer Institute data for cytomegalovirus many years ago. They found it in a large group of patients and then didn't find it afterward. Did the Stanford University group find an increase of any other virus disease such as cytomegalovirus? Secondly, what is the mechanism of dissemination? If local dermatome involvement occurs by retrograde spread from the nerve, how does herpes become disseminated in these patients? Is it via the bloodstream, or local contact, or neurologic?

Stevens offered to reply to the second question first. He believed viremia is clearly the mechanism of dissemination because the number of pocks and the rapidity with which they appear suggest that it could not be neural spread involving so many different sites simultaneously. So he thinks it is a viremia which originates in the initial site of virus infection. Some of the more severely involved patients have large dermatomes involved, with sloughing vesicles, and some patients have even required skin grafts. There is a lot of virus replicating there, and he thinks that is the site which seeds. As far as other infections are concerned, candidiasis and cryptococcus are 2 infections of high frequency in the Hodgkin's patients. He thought there had also been a few cases of cytomegalovirus, including some which involved the lung, but could not give any incidence figures on those diseases. **Rosenberg** asked whether there was a corresponding increase in these other infections. It sounds like something very peculiar to herpes and not just a generalized disturbance. **Stevens** said that he and Doctor Merigan had reviewed this subject a few months ago, and it was their impression that certain virus groups are more associated with immunosuppression, particularly with defects in cell-mediated immunity. Those are the poxviruses and the herpesviruses. There does not seem to be either an increased incidence or more severe disease with other agents, including the respiratory viruses and enteroviruses, though there are isolated cases where perhaps one sees a slightly increased incidence of severe complications with those viruses. However, the poxviruses (in man, vaccinia is the main example) and the herpesviruses (herpes simplex, cytomegalovirus, and varicella-zoster) are all severe in Hodgkin's disease patients, in renal transplant patients who are receiving azathioprine and prednisone, and in patients who have congenital or acquired defects in their cell-mediated immunity function. There seems to be a common thread which involves those virus groups as well as the fungi and the *Pneumocystis* organism.

Carbone questioned Doctor Stevens again whether it doesn't seem peculiar that in this patient population there has been no unusual increase in these other infections comparable to that of herpes zoster. **Stevens** replied that varicella-zoster infection is very apparent because it occurs in the skin, whereas cytomegalovirus may be the cause of occult fever and of abnormalities in the urinary sediment which may not be detected; if the disease resolves or smolders without any fatal sequela or visceral involvement, it may not be recognized. We have a high probability of diagnosis of varicella-zoster because it is right in the skin. **H. S. Kaplan** asked Doctor Goffinet about the possible influence of more extensive radiation techniques. He pointed out that another factor changing over the time period that Doctor Goffinet had analyzed was that total-nodal radiation was used more regularly. For example, by late 1967 the L-1 protocol data on the Stage I-B and II-B cases had become evident. As a result there had been a shift over to total-nodal radiotherapy as the routine treatment for many more patients. He wondered whether Doctor Goffinet had a breakdown of the nonsplenectomized patients as well as in those subjected to splenectomy for the incidence of herpes zoster in patients who received total-nodal radiotherapy versus those who received only local or extended-field radiotherapy.

V. T. DeVita stated that the incidence of herpes zoster-varicella after MOPP chemotherapy (mechlorethamine, vincristine, procarbazine, and prednisone combined) is exactly 10%. He asked Doctor Stevens whether by dissemination he meant the appearance of 10–20 lesions. This is really quite common. Many people just overlook this and fail to score it as dissemination.

Stevens replied that the incidence cited by Doctor Rosenberg from a published large series of cases had greatly relied on chart review, which may underestimate the incidence of dissemination. As Doctor DeVita said, there are many patients who have 20 vesicles which occur during a short period. They only have 2 days' worth of dissemination and they may not notice it. They do not have visceral involvement, and they may not report the added vesicles to the doctor. Most series have reported anywhere from 30 to 50% dissemination so, although the incidence is higher at Stanford University, he did not think it is drastically higher. Possible factors which may be influencing the dissemination rate at Stanford University include the total-nodal radiotherapy, MOPP, and splenectomy.

E. Frei asked whether there was any relation between the sites of Hodgkin's disease and the dermatomes involved by zoster. **Stevens** said he had seen the previously described association of an excess of dermatomal involvement in the areas of prior radiotherapy and also of tumor involvement. It is impossible to separate those 2 factors. **D. R. Goffinet** added that approximately two-thirds of the Stanford University patients who developed herpes zoster had Stage III or IV Hodgkin's disease, but that the longest dermatomes seem to be the ones that have the most viral infections, as one would expect.

In answer to **Kaplan's** question, he was not able to break the data down by the year, but there were 22 patients who received local radiotherapy and developed herpes zoster out of 122 total cases of herpes zoster in the entire group.

Part 4. Panel Discussion: Approaches to Immunotherapy of Hodgkin's Disease

Chairman: G. Hamilton Fairley

SUMMARY OF INFORMAL DISCUSSION BY THE PANEL

K. E. Hellström stated that evidence is ample today that experimental animal tumors have specific antigens and evidence is now forthcoming from many laboratories that human tumors are antigenic also. The nature of these antigens in the human tumors is so far somewhat unclear. Some of these antigens might be tumor specific. Many of them, maybe most, are tumor associated and may also be associated with certain normal cells, at least with normal embryonic cells. Of course, the fact that a human tumor has certain antigens does not exclude the possibility that it can have other antigens in addition to those. Evidence is rather clear that the malignant melanomas do have common antigens in addition to individually unique antigens.

Now, about the nature of immune reactions to these antigens, the common concept is that the most important arm of the immune response is the cell-mediated type of immunity, which is mediated by immune lymphocytes and by macrophages. The lymphocytes have been shown with a variety of assays in tissue culture to be cytotoxic to the tumor cells. The exact mechanism of the cytotoxic reaction is unclear. Lymphocytes seem to be needed to establish close contact with the tumor cells that they kill but, under certain conditions, humoral mediators such as lymphotoxin leak out into the medium that can kill the tumor cells at a somewhat wider range. Macrophages can also kill the tumor cells by a direct cytotoxic effect.

What can serum factors do? Under certain conditions, factors present in sera of tumor-bearing animals and people can block the cytotoxic effect of specifically immune lymphocytes. These blocking factors are in most cases at least partial antibodies and have, therefore, been referred to as blocking antibodies. There are good reasons to believe that antigens often combine with these antibodies to form blocking antigen-antibody complexes and, under certain conditions, antigen alone present in the serum may block. This blocking effect seen in vitro corresponds to the phenomenon of immunologic enhancement in vivo. Recent experiments showed that such blocking sera were also active when given in sufficient amounts to rats in vivo. These rats when tested again had a blocking serum activity. Moreover, tumor cells implanted into such rats were enhanced; i.e., they grew better than they would under control conditions. Thus, serum factors can enhance tumor growth and block cell-mediated immunity.

That does not mean that all kinds of humoral antibodies are bad or that we should try to use antiplasma serum to get rid of all humoral antibody. There are other antibodies in the serum that could be quite helpful. For example, cytophilic or opsonizing antibodies can help in arming macrophages so that they become activated to react against tumors; cytotoxic antibodies can kill tumor cells in vitro together with complement. Finally, to make it even more complicated, Hellström's group has recently been interested in what they call "unblocking" antibodies, which may or may not be the same as the cytotoxic antibodies. These antibodies are immune molecules with tumor specificity that can abrogate the blocking effect of serum that one sees in animals with tumors and in certain cancer patients.

Now, if one wants to do immunotherapy in Hodgkin's disease, where should one look? What one would really want to know would be the effect or mechanism of tumoricidal reactions in vivo. The fact that lymphocytes kill tumor cells by close contact in vitro does not necessarily mean that this is the most common mechanism by which an antigenic tumor is reacted against in vivo. There is evidence that such lymphocyte-mediated reactions in vivo are quite important: If we give antilymphocyte serum, for example, to depress the activity of thymus-dependent cells, we get better tumor growth. But the interaction between lymphocytes and macrophages and various serum factors in vivo is not fully understood. So, we have then to make relatively simplistic models to come up with suggestions that would possibly be worthwhile. One possibly worthwhile step would be to improve upon the cell-mediated immune reactivity. This could be done nonspecifically by giving bacillus Calmette-Guérin (BCG) and similar substances. It could be done by attempting vaccination, by vaccinating, e.g., intradermally, since certain studies indicate that that is a particularly good immunizing route. It could be done by vaccinating with slightly modified antigens, or by giving RNA from specifically immune lymphoid cells, by giving so-called transfer factor, or by giving selected serum materials or plasma. If one can find serum or plasma that would have good cytotoxic activity on tumor cells in vitro or unblocking activity or contain cytophilic antibodies, that could be helpful. The problem has been that such serum could have adverse effects; the serum could give the enhancement phenomenon, which would be bad for a patient, rather than to give unblocking or cytotoxic effects, which would be good for the patient. The availability of in vitro assays to monitor such reactions is quite important if one wants to use anything of that sort.

What is the defect in patients with Hodgkin's disease? We know very little about cell-mediated immune reactions to Hodgkin's target cells, but from the little we know it seems that anergy in patients with Hodgkin's disease plays a much larger role than in a variety of other human tumors. On the other hand, so far it has not been possible to detect any blocking serum effect. Thus, what seems to be needed would be to improve upon the cell-mediated immune reactivity to the Hodgkin's target cells and to find out more about why patients are anergic and what could be done to overcome this anergy. To do the in vitro studies, one obviously needs better target cells than what have been studied so far. Maybe some of these cell lines that Doctor Sanders and others have been working with can be of value to study this further. We should certainly realize that even in the animal systems in which we know well that we have tumor-specific antigens that we can characterize much better than in the human situation, the effect of immunotherapy has not been overwhelming. There is still not much knowledge about spontaneous or chemically induced primary tumors in animals that are models for human tumors and where by giving immunotherapy one has been able to cure the animals. Those systems that have been studied have been fairly artificial, but, on the other hand, by using such artificial models we are beginning to understand a little bit better what can be done. So far Hellström had been relatively unimpressed with what he had seen of human tumor immunotherapy. There are some reports of striking responses but very few if any situations in which one can really draw firm conclusions about an immunotherapeutic effect. Hopefully, properly controlled experiments done on a double-blind basis with random selection of patients will yield conclusive answers.

G. H. Fairley believed that, although all the animal systems used are artificial, under a number of circumstances one can alter the course of animal malignant disease by various forms of immunotherapy. In the past, the great difficulty has been that one has needed such a powerful immune response to destroy even a few malignant cells that it has not looked a very feasible proposition in terms of the total numbers of malignant cells there are likely to be in man at the time one must treat the disease.

I. Weissman said he was glad that Doctor Hellström had given an introduction to this subject and had talked about his data indicating that, in the animal models and in the human tumors studied to date, there does seem to be a blocking effect by some factors in serum of the host's cell-mediated immunity. Therefore, one is forced to look for a model which will circumvent this. Obviously one cannot approach immunotherapy when such factors are present in the serum of the patient, whether the blocking is due to antibodies that mask cell-surface antigens of the tumor, or antigen-antibody complexes, or free antigen. One eventually is going to have to develop a system that can diminish the undesirable factors and increase the beneficial factors. One approach that his group has been trying is to render antibodies cytotoxic. One can imagine an antibody molecule which has a couple of combining sites for antigen at one end. Infused into an animal bearing a tumor that normally would be rejected by a cellular immune response, such an antibody will reverse that rejection. That is the phenomenon which in animals has been called immunologic enhancement. Now, let us try to make that a cytotoxic antibody instead of an enhancing antibody. The obvious thing is to try to get some cytotoxic influence to adhere to that antibody. Then we could let that antibody localize to the tumor and when it had done so, the adherent agent would cause a cytotoxic effect. One can think of a number of factors that might be cytotoxic. Perhaps the most efficient would be an enzymatic effect, i.e., an enzyme that would adhere to the antibody, then react with the surface of the cell, and punch a hole in the cell membrane. There is really no better enzyme for that purpose than our complement system, which does just that. Yet, in animal model systems and probably in human tissue, complement is insufficient to do away with most tumors. Another category of toxic substances is radioactive isotopes. About 10 or 15 years ago, Eugene Day and David Pressman began a series of experiments in which they tried to radioiodinate antibodies and use these as potential therapeutic agents in animal tumor models. It is very simple to use carrier-free iodides to obtain iodination of antibodies. It is an oxidation reaction which has an oxidized iodide intermediate. They used chemical oxidizers in fairly high concentration. There was never a very careful study done on how little of an oxidizing agent one could use. They found that, after they got to an average of about 3–5 iodides per antibody molecule, they started drastically losing antigen-binding capacity of the antibody molecules, as if either the iodination or the oxidation somehow affected the antibody-binding site.

Could one hope to get enough iodides on antibodies so that one could at least theoretically develop a system that might be either diagnostically useful or therapeutically effective—i.e., that would deliver a dose of irradiation to a microscopic focus that might be sufficient to therapeutically ablate the tumor? Are there sufficient antigenic sites on the tumor? Does the tumor cell membrane turn over too fast for antibodies to stay bound? How many iodides per antibody are necessary for either diagnostic detection of microfoci or therapy of microfoci? Obviously if one ever tried to take this system back in vivo, one is going to get into a lot of problems with circulating antigen or circulating antibodies. That is why the first step is to study the problem in vitro.

These are some of the critical factors. External detection is only feasible with gamma emitters, and the detection is a function of the total deposited iodide load in the local area compared to the background. It depends on the number of iodides, no matter what size the focus is, whereas thereapeutic effect is not a function of the total number but of the concentration, i.e., the number of iodides per cell no matter whether the focus is one cell or a million cells. Therefore, among the critical factors that one must consider are the number of cells per focus, the number of antigenic sites per cell, the half-life of the cell membrane, the specificity of the antibody, the avidity of the antibody, the total number of iodides per antigenic site, and the vascularity of the tumor.

Equations can be derived to estimate the total dose of radiation delivered to a tumor focus by an antibody bound to it. Using the techniques that Doctors Johnson, Day, and Pressman developed to bind about 1 iodide per antibody and making the assumptions that there are about 1,000 tumor antigenic sites per cell, and that the effective half-life of the antibody on the cell membrane is about an hour, one can compute the limiting volume and number of tumor cells that could be detected. For comparison, one can recall that a 1-cc volume of tumor containing about 10^9 tumor cells is about the limit of detection for conventional radiographic procedures such as lymphangiography. With radiolabeled antibody under the conditions described above, one might be able to detect as few as 10^5 or 10^6 cells, but the dose delivered to them by the ^{131}I would be only about 33 rads.

The possibility was tested that the actual concentration of the oxidizing agent might be more important than the number of iodides put on in inactivating antibody binding to antigen. Using a low concentration of oxidant which was sequentially replenished as it was reduced in the reaction, one can add substantially more iodides per antibody, as compared to the old high oxidation technique, without reducing the antibody titer. But even with that technique one cannot go too high before one starts inactivating the antibody. A discovery by Doctor Morrison at City of Hope led us to the use of enzymatic iodination. Enzymatic iodination has the theoretical advantage that an enzyme with a catalytic site for iodination may not be able to penetrate the region of the antigen-binding site of the antibody. Thus, one might get a shell of iodides added in areas of the antibody that do not compete with its antigen-binding function. With the use of Moloney virus-induced lymphomas and sarcomas in mice, radiolabeled antibody was prepared by Morrison's lactoperoxidase technique. There is no decrease in the ability of the antibody to bind with the lactoperoxidase technique at limiting concentrations of the oxidizing agent, which is hydrogen peroxide. This allows one then to get much higher concentrations of ^{131}I or ^{125}I on the antibody molecule.

Studies of the kinetics of binding and detection with such lactoperoxidase-iodinated antibody show that one can theoretically detect very small numbers of cells even with lightly labeled antibody. Using carrier-free ^{131}I and maintaining the same statistics of binding that one can now expect in vitro with this system, one could detect on the order of tens to hundreds of tumor cells, and one could hope for a radiotherapeutic dose of $>$33,000 rads delivered to these cells. Of course, this has to be verified in an in vitro model system before one can hope to apply this approach in animals and perhaps later in man.

S. E. Order stated that, regardless of the methodology used—and there are many methods being employed—the secret of success in the use of immunology both diagnostically and therapeutically is specificity. We must be able to demonstrate that the target site which we want to derive antibodies or lymphocytes to react against is specific. In Hodgkin's disease, it becomes particularly confusing because none of us knows exactly what cell should be the target.

Order presented some of the studies his group had done and tried to relate them to the cell infiltrate in Hodgkin's disease. Earlier in the week, he had reported on their data concerning at least 2 antigens. By immunoelectrophoresis, they could detect these 2 antigens using heterologous, absorbed, rabbit antisera against the Hodgkin's tumor infiltrate. One antigen is a fast migrating antigen. They could detect it in 22 of 23 splenic tumors from patients with Hodgkin's disease. Interestingly enough, the fast migrating antigen predominates in the spleen over the slow antigen. The fast antigen was also found in quite high concentration in some of the presumed autoimmune diseases. It was also found in embryonic tissue, particularly in the fetal liver. The slow antigen was found in the lymph nodes predominantly in 5 of 6 cases with Hodgkin's disease. It was also found in the very earliest fetal liver at 8 weeks of gestation. Finally, both the slow and fast antigens were found in a neonatal thymus but not in fetal thymuses.

They then began to launch into more specific methods of measuring these same antigens. Protein characterizations were carried out on the splenic tumor infiltrates of some of the Hodgkin's disease patients. Similar curves have been obtained with one lymph node. There is a sharp protein peak seen when the material is run through a G200 column and the fast aliquots are removed, and then there are two smaller peaks that follow. When normal spleen antigen was run in immunoelectrophoresis with the absorbed antisera, there was no reaction. The fast aliquot from the G200 Sephadex column has exactly the same specificity as the whole tumor, whereas the second and subsequent aliquots gave no reaction. So it seems that the fast antigen is in the fast protein peak and this is a great advantage, because that protein peak is at very high concentration. An estimate of that protein peak in one particular case was 660 mg protein per ml of starting material.

They also used other technologic means, such as a discontinuous bovine serum albumin (BSA) density gradient, which segregates Hodgkin's disease tissue into 4 cellular layers. The cells they obtained in Layer A of a BSA discontinuous gradient preparation from a patient with lymphocyte-depleted Hodgkin's disease included a mixture of large cells, medium-sized cells, and some smaller cells. These cells segregate on the basis of their density. Both Layer A and Layer B, the least dense layers in the spleens studied so far, seem to contain the fast antigen component. This approach allows one to dissect the cellular populations because the BSA gradients can be changed so that one separates the cells in different manners, or one can take a given layer and separate it by a second BSA gradient run. It should thus be possible to produce purified antisera to the 2 antigens. Secondly, it should become possible to see what cells are involved by immunofluorescence once the purified antisera have been produced.

Meanwhile, **S. Hellman** and he had some thoughts derived both from their experimental work and from clinical experience which suggested a hypothesis about the pathophysiology of Hodgkin's disease.[1] Doctors Kaplan and Smithers had originally suggested in 1959 that Hodgkin's disease is an autoimmune disease involving a form of graft-versus-host reaction. The new hypothesis takes that as a point of departure and attempts to delineate the actual cell-to-cell steps which may be occurring in the genesis of Hodgkin's disease. They visualize the following sequence. Lymph nodes contain T-cells, the thymus-derived lymphocytes involved in cell-mediated immunity. A virus or viruses gain access to the area occupied by T-cells in the lymph node, spleen, or thymus. These are regions where T-cells are known to be concentrated and these are also the regions commonly associated with the focal occurrence of Hodgkin's disease. Is there a model for viral infection of T-cells? In nature there are specific, well-studied models, the most well known of which is the TL system of L. J. Old and E. A. Boyse who described viral infection of T-cells during the repopulation of the thymus in irradiated mice before developing leukemia. So it is not farfetched to postulate that viruses may infect T-cells selectively. If T-cells do get infected by a virus, some of them may undergo antigenic conversion of their surface. This type of antigenic change has been documented in a wide variety of circumstances. At some point in time, therefore, in the lymph node or the spleen or some other focus, immunocompetent T-cells may be looking at antigenically altered T-cells and begin to react immunologically. If one takes a patient's lymphocytes and grows them in culture, one can produce lymphoblasts. If one then inhibits these lymphoblasts with mitomycin C so that they cannot divide, one can place the same patient's fresh lymphocytes in the same culture and show that the DNA uptake of those lymphocytes is quite high, suggesting an autoimmune reaction is occurring in which lymphocytes of the patient are reacting against his own lymphocytes which have been maintained in culture. Furthermore, it has been reported that the same kind of event is seen in infectious mononucleosis when fresh autologous lymphocytes are later used against the patient's own atypical lymphocytes cultured during the active phase of mononucleosis.

If T-cells interact against other T-cells in this way, such reactions may well explain T-cell depletion in Hodgkin's disease, as well as the lack of delayed hypersensitivity. This type of T-cell–T-cell interaction is also seen in graft-versus-host disease when the strains are coisogenic or very closely related. Doctors Schwartz and Beldotti, a number of years ago, showed that they could induce graft-versus-host disease in mice and produce lymphomas with greater rapidity. During the course of the graft-versus-host disease, they also noted that abnormal reticulum cells are produced, so once again nature has provided us with a model consistent with this possibility. Finally, of greater interest is the fact that the number of lymphocytes the patient has in his local tumor or, in other words, the pattern of lymphocyte predominance versus lymphocyte depletion in Hodgkin's disease seems in some way to affect his general prognosis. One might wonder whether some kind of abnormal lymphocyte which has been antigenically altered may not be an initiating factor. Once again, this is a hypothesis, but many things could be measured in relationship to this hypothesis to help develop a constructive view. In the immunology of Hodgkin's disease, we are in the stage of description, not of therapy. We must clarify the role of the various cells involved in the infiltrate so that we can more clearly understand the sequence of events.

D. Wright asked how this hypothesis would account for the bimodal age-specific incidence curve in Hodgkin's disease. **Order** commented that this might relate to the epidemiology of exposure to the hypothetical virus(es). The epidemiologists earlier in this Symposium showed that it also could be socioeconomic in its relation—that it was the very poor classes and very young people that are affected. There is also probably more than one virus involved. If one looks at lymphocytes as having an effector mechanism which can be turned on by a variety of stimuli, then viruses may be one of the blastogenic factors. One other point is that, as we get older, we all become T-cell depleted. This is a progressive process over the years as more antigens challenge our system, because the T-cell population is not an infinite population. With T-cell depletion, susceptibility to tumor development is increased. In an experiment published by Doctor Cornelius at Yale, in which just the thymus was removed from animals very susceptible to a lymphoma, a high incidence of lymphomas resulted.

We have looked at lymphocytes in the past and said they were lymphocytes, but this is no longer justifiable by today's standards. There are at least 5 known antigens on the surface of animal lymphocytes. This might well be relevant to the question: "Why is HL-A5 associated with Hodgkin's disease?" The TL+ antigen is related to the *H-2d* locus in animals which is a histocompatibility antigen; TL+ is a tissue antigen and LYA and LYB are lymphocyte antigens that have been described and are related to specific genetic loci. If tissue antigens are related to specific loci and if HL-A5 represents histocompatibility, is there a relationship between the tissue loci and the histocompatibility loci which could relate to susceptibility? In the TL+ system that happens to be true, but we do not yet know much about the tissue specificities in man.

H. S. Kaplan stated that about a year ago he had presented a very similar modification of the original Kaplan-Smithers' hypothesis at the British Association for Cancer

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[1] ORDER SE, HELLMAN S: Pathogenesis of Hodgkin's disease. Lancet 1:571–573, 1972.

Research meetings.[2] It was not adorned with modern T-cell terminology but otherwise was substantially similar.

There is really no proof that Doctors Schwartz and Beldotti or anyone else had ever induced a neoplasm with graft-versus-host reactions. The mice in which these tumors appeared are prone to develop a little later in life exactly the same type of tumor without any such treatment. If one has only accelerated the onset of a tumor which would otherwise occur, could the whole thing really be due to immunosuppression, resulting from the graft-versus-host response, causing lymphocyte depletion followed by the expression of a latent tumorigenic virus, which has been there the whole time, but which has been kept in check by the immune state of the organism? This is at least a reasonable alternative hypothesis in this situation.

Order agreed, but suggested that there may be some direct clinical evidence in chronically immunosuppressed patients receiving kidney transplants who developed both lymphosarcoma and reticulum cell sarcoma. He knew of no case yet of Hodgkin's disease. However, a variety of models shows that antigenic stimulation with concomitant immunosuppression does lead to lymphomas.

E. Hersh stated that he and his group had not treated any patients with Hodgkin's disease with BCG. However, they had started to accumulate some experience in the treatment of malignant melanoma with BCG. Their data to date suggest that one of Doctor Hellström's proposals is possible, i.e., that one might be able to increase the level of cell-mediated immunity to a variety of antigens with this material. They have attempted to select a group of patients with malignant melanomas who might be particularly susceptible to benefit by nonspecific immunologic stimulation. This is the group of patients who have had a primary tumor in the trunk and have also had regional node metastases with at least 5 positive nodes at some time subsequent to the removal of their primary tumor. These patients generally have relapses within about a year (>75% of patients). It was thought that by administering BCG to such patients when they had no evidence of disease and presumably a very much reduced tumor burden, one might be able to detect within a year or so some clinical benefit. They had started this program only about 5 months ago and did not yet have any data to indicate therapeutic benefit.

However, they do have some interesting immunologic data. Their objective of course was not only to prolong the relapse-free interval in these patients but also to see if one could modify cell-mediated immunity. The patients all underwent an extensive work-up to rule out the presence of evident metastatic disease, including a complete radiologic evaluation, brain and liver scans, and hematologic and bone marrow evaluation. They then underwent immunologic evaluation including lymphocyte count, hemoglobin levels, the lymphocyte blastogenic response to such agents as phytohemagglutinin (PHA) and tuberculin, and delayed-hypersensitivity skin testing with a battery of 5 antigens. This battery of tests was repeated monthly after the administration of BCG, but the skin tests were done just once—4 weeks after the start of the treatment. BCG was obtained from the Tice Laboratories in Chicago and used in 2 dose schedules, a low-dose schedule of 7.5 mg, about 1×10^7 organisms, and a high-dose schedule of 75 mg, about 1×10^8 organisms. They used the scarification technique, exactly as Georges Mathé has described in his papers on this subject, and administered the BCG either every 4 or 7 days for the 1st month, depending on how frequently the patient could come back, and then monthly thereafter.

Results from delayed-hypersensitivity skin testing are available in 20 of the 22 patients treated between October 1971 and late February 1972. The BCG treatment has been extremely well tolerated by the patients, who had no outward side effects of any kind, including no severe or intolerable local reactions and, in particular, no disseminated BCG disease, which is, of course, a serious concern in patients who are somewhat immunosuppressed. Among the 20 patients capable of being evaluated, 4 were anergic to the battery of 5 skin test antigens. In addition, they were tuberculin negative, so they were actually anergic to 6 antigens. All 4 of these patients had a reappearance of delayed hypersensitivity to at least one of the antigens in the battery and developed positive skin test reactions to tuberculin itself. Thus, as a result of BCG treatment, all 4 anergic patients became allergic. In addition to that, 9 of the other patients had significantly increased skin test reactivity. That is, they either had an increased number of positive skin tests or one of the skin tests that was previously positive increased at least 100% in diameter of induration. No patients had a significant decrease in their skin test reactivity, defined as a diminished number of positive reactions or a >50% decrease in size of the skin test reactions. All 20 of these patients have developed tuberculin reactivity.

In all lymphocyte cultures from these patients, the DNA uptake responses to PHA tended to increase, sometimes as much as twofold to threefold. However, the baseline, unstimulated lymphocyte culture reactivity also increased, so that the stimulation index (the stimulated culture counts divided by the unstimulated culture counts) did not really increase. They have not yet analyzed the immunoglobulin data.

In essence, then, they have observed that 60% of these patients have shown definite improvement in their skin test reactivity. One might ask what a similar group of untreated patients would do. Indeed, as we all know, skin test reactivity responses of cancer patients fluctuate. They have about 25 untreated melanoma patients in good condition with little or no disease whom they have studied. In that group 10% improved and 10% declined in their reactivity in a similar interval without BCG treatment. With further study, this will probably turn out to be a significant difference. None of the 22 BCG-treated patients have had a relapse to date. However, it is too early to tell whether there has been a significant therepeutic effect on their melanomas. At any rate, these data do suggest that the immunologic reactivity of these patients has been stimulated, in terms of improved delayed hypersensitivity, through a tolerable form of treatment.

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[2] Kaplan HS: Role of immunologic disturbance in human oncogenesis: Some facts and fancies. Br J Cancer 25:620–634, 1971.

Hellström asked whether there was any evidence of altered delayed-hypersensitivity reaction to specific melanoma antigens in these BCG-treated patients. **Hersh** replied that he and his group are most interested in that question. They have tumor stored away from the time of surgery on only 2 of these patients and do not have any data yet as to whether their reactivity is improving. The other approach that they are attempting to use is a cytotoxicity assay with the patients' lymphocytes against the melanoma line which they have been growing in vitro. They have been having some technical difficulties, so they do not have any positive data there either, but they have this approach definitely in mind and plan to use it as soon as they can get the appropriate test system working.

P. P. Carbone mentioned that B. G. Leventhal at the National Cancer Institute has been doing this in leukemic patients. Thus far, she has shown no evidence of enhanced tumor response to the leukemic cells in vitro. In fact, in a control study in which she randomly allocated patients to either immunotherapy or to therapy with methotrexate, the group given methotrexate, if anything, had a better response to their tumor cells than the other group.

The same goes for Burkitt's lymphoma in which J. Ziegler has about 12 patients who have been randomly allocated to either BCG treatment or no BCG treatment. They have shown no evidence that specific immunity has been enhanced by BCG. The curious thing is that the site of relapse is different for the 2 groups, but this may not be significant. The patients getting BCG tended to have relapses in the central nervous system, whereas the others did not. **Hersh** pointed out that it is blastogenesis which Doctor Leventhal is measuring. A phenomenon which a number of workers have described is increased or "rebound" reactivity when intermittent chemotherapy is given and when one measures the response several weeks later. However, we are not going to find the same type of immunotherapeutic benefit in every malignancy and that is the critical reason Doctor Hellström suggested that this approach is so important. **Hellström** commented that there is some reported therapeutic effect of BCG in experimental animals, specifically, as tumor regression in rats. It is important to reduce the tumor mass as much as possible before BCG is given. With large tumors, one can get an increase in blocking antibody and enhanced tumor growth. **Hersh** concurred, saying that this is a very important point that many people have made in discussions of immunotherapy: The tumor burden has to be minimal. That was the basis for selecting the particular group of patients to start their immunotherapy tests.

J. H. Frenster opened his part of the panel discussion by returning to the original question: Is immunotherapy going to be a possible form of treatment in Hodgkin's disease? Some of the original optimism concerning immunotherapy derived from pathologic studies which suggested that there is more to a tumor than a neoplastic cell population—that often there are reactive elements such as lymphocytes and plasma cells also found within the neoplasm. There have been several interesting correlation studies in Hodgkin's disease, in carcinoma of the breast, in carcinoma of the stomach, and in other cancers. These studies have indicated that the more lymphocytes one finds in the original surgical biopsy before the patient has been treated, the better the prognosis. It is now recognized that in many human neoplasms there are surface antigens on the neoplastic cells which often can be found in free form, sometimes circulating in the blood of patients. Hellström's beautiful assays have shown the presence of sensitized lymphocytes specific for tumor cells of the patients who donated the lymphocytes. There are evolving data that free antigen can be deleterious to patients, perhaps by virtue of inactivating the sensitized lymphocytes, either as free antigen or in combination with 19S or 7S blocking antibody. Looking at the interaction between what may be the malignant cells in Hodgkin's disease, whether derived from reticulum cells or from T-cells via viral transformation, and the immune elements, we can recognize several important parameters, such as the presence of tumor antigen which might sensitize the T-cell system and the effects of excess tumor antigen in such T-cell-mediated lymphocyte immunity. We now recognize that we have at least 2 lymphocyte populations—the T- or thymus-dependent and the B- or thymus-independent, the latter giving rise to antibodies which may either be cytotoxic or blocking and the former giving rise to killer lymphoblasts which effect rejection and are presumably important in tumor rejection. It has been recognized that specific antigen is the proper source for transformation of such T-lymphocytes to the killer form. However, it is not essential to use specific antigen; instead, one can take advantage of the blastogenic properties of PHA. PHA has been shown to be fairly specific for activation of the T-lymphocyte system and to have little, if any, effect on the B-cell system. He and his coworkers have taken advantage of that phenomenon to ask the question whether there would be any value in specifically activating the T-lymphocytes in tumor-bearing patients. At this point they have not studied Hodgkin's disease patients but have worked with other patients bearing tumors. The rationale has been as follows: There is an obvious inability of unstimulated lymphocytes to control the growth and spread of neoplasms, and stimulation by PHA of the lymphocyte reaction against autochthonous neoplastic cells is weak but can be shown to be of definite effect. That is, if one compares the value of adding lymphocytes to target cells in vitro before and after PHA stimulation, there is a definite enhancement of such cytotoxicity after PHA activation. However, we cannot use PHA directly in vivo because it in itself is an antigen and results in the development of the antibody very soon after it is injected. As a consequence of these constraints and potentialities, they had initiated a study of patients with far advanced neoplasms who were relatively asymptomatic and could be hospitalized on an ambulatory basis for 1 week each month and did not require chemotherapy or any other form of therapy concurrently. They harvested whole blood from such patients and separated the leukocytes by low-speed centrifugation, reinfused the erythrocytes within 1 hour so that the patient would not develop any significant anemia, and placed the resultant lymphocytes that were obtained in culture with highly purified PHA for 48-hour periods, changing the medium at 24 hours and then washing extensively at 48 hours before reinfusion into the patients. One can then observe the usual blastoid transformation in vitro. They soon encountered problems related to the quantities of lymphocytes that could be harvested. In a 70-kg person, it was difficult to isolate $>10^9$ lymphocytes. During leukocyte separation, activation, and reinfusion, they ended up with 15% yields. They have subsequently been able to improve this to approximately a 50% yield. They hope to be able to increase the number of lymphocytes harvested by mechanical techniques involving the blood cell separator.

He would like to confine the discussion of their clinical experiences to those patients who have had at least 10 reinfusions of activated autologous lymphocytes in a 3-month period. There have been 5 such patients in the study so far who had received between 10^8 and 10^9 activated lymphocytes per infusion for a total of at least 10 reinfusions. Unless premedicated, patients demonstrated minimal pyrexia lasting for up to 4 hours after the conclusion of the 1-hour reinfusion. This could be moderated by aspirin pretreatment or more extensive washing of the cells at 24 and 48 hours. There have been no instances of thromboembolism and pulmonary infarction, hypertension, hematologic complications, and allergic phenomena on repeat reinfusions.

The 5 patients include 2 with generalized malignant melanoma, 1 with Ewing's sarcoma, 1 with embryonal cell carcinoma, and 1 with metastatic carcinoma; all of these patients received no concurrent therapy for at least 3 months before the reinfusions. Three of these patients have shown objective regressions of $>50\%$ of lung nodules on serial chest X-rays lasting for at least 1 month following such infusions.

Some of the problems of the procedure relate to the numbers of lymphocytes they have been able to deliver. One would like to have at least 1 activated lymphocyte for each tumor cell. When one calculates the numbers of tumor cells present in these patients, one is sobered by the realization that even a small nodule, a 1-cm diameter lung nodule, may contain 10^8 tumor cells 20μ in diameter. They would like to increase the number of lymphocytes and they hope to do this via continuous flow centrifugation. They would also like to render the lymphocyte activation procedure more easy and rapid.

In a parallel series of experiments, Doctors Cheema and Hersh have followed a similar procedure of lymphocyte activation with PHA and are now returning these cells intracutaneously to patients who have cutaneous metastases of more distant neoplasms. They compared the effect of autologous PHA-activated lymphocytes with that of autologous lymphocytes incubated but not activated, with that of saline injections, and with no injection. They related the number of lymphocytes injected to the volume of the tumor. Observing the patients for up to 28 days and scoring the regressions between 9 and 28 days following these injections, they could demonstrate a selective effect of lymphocytes and a greater effect when those lymphocytes had undergone prior PHA activation.

In another approach, he and his colleagues had been looking at the question of whether the maximal dose of drug that can be given for animal tumors or that we might use clinically corresponds to the optimal balance between the therapeutic and immunosuppressive effects of the drug. There were suggestions earlier in the literature that, if one relates drug dosage to the tumor suppression and immunosuppression one can observe in either animal or human systems, then because of the rather low growth fraction in tumors there is often a plateau effect of high doses of drugs with respect to therapeutic effect and their immunosuppressive effect shows less of that plateau. This could be of great importance in the clinical situation in which there is some immunity opposing tumor growth. When one uses a high dose of drug, the degree to which one gets more immunosuppression than tumor suppression might in fact be doing the patient a disservice by that extremely high dose of chemotherapy. If in fact there are drug dose levels at which tumor suppression is greater than immunosuppression, that might be a more optimal situation. They are beginning to look at this question clinically and have deliberately reduced their doses of drug combinations in patients with a variety of neoplasms to levels approximately 30% to 50% of those usually given to such patients. To date, they have studied 17 patients, 11 of whom have completed 1 month of therapy. One of these is a 32-year-old woman with a 2-year history of a carcinoma of the cervix metastatic to the lungs with a huge right lower quadrant mass, 10×10 cm, that had given her a lumbosacral plexus paralysis and an inability to extend her hip. She was placed on low-dose combination chemotherapy. Three months after continuous therapy, the size of all nodules in the lung was markedly reduced. By that time, the 10×10 cm mass had disappeared and she was fully ambulatory. Three months later, only 2 small lung nodules were left in this patient who now has gained 25 pounds and is living a normal life. Of the first 11 patients who have completed at least a month of therapy, 7 have shown objective regression of disease of $>50\%$ for >1 month, 2 have stabilized their disease which had previously been progressive, and 2 have continued to progress despite this therapy.

It is clearly difficult to deal with neoplastic processes by immunotherapy alone. It will probably be necessary to combine immunotherapy with other modalities. For disseminated disease, it will be necessary to consider some form of chemotherapy which will be compatible with immunotherapy. Such compatibility will require that there not be significant immunosuppressive effects and, more importantly, suppression of the lymphocyte counts by the drugs, used at optimal dose levels.

Hellman pointed out that one must be very careful with comparisons of curves with complex shapes. The curves shown by Doctor Frenster suggest that there is a different shape for the drug dose-response curve for tumor cell response versus immune response, that the curve for chemotherapeutic response showed a resistant fraction. There is no reliable evidence that that always occurs and there is really fairly good evidence that a resistant fraction may not occur. Without going into details, the point is that response is going to be very different for each tumor versus the normal tissues and in some cases the relationship to dose is going to be quite the reverse.

Frenster replied that that certainly could be determined experimentally. George Hahn has currently tested only a limited number of drugs in the C3H sarcoma system. He is able to look concomitantly at immunity in that system, in which he sensitizes the animal specifically to the tumor and then employs the drug used for tumor chemotherapy as an immunosuppressant and can relate the quantity of cells necessary to produce a tumor in vivo as a function of both the immunized state and the chemotherapy dosage.

E. Frei asked whether the PHA-activated lymphocytes are swept out of the circulation in the lung. **Frenster** responded that he had seen liver disease progress in the face of regressing pulmonary nodules so that is entirely possible.

Fairley opened his discussion by referring to the situation in acute leukemia with regard to immunotherapy, since Doctor Kaplan had asked him to give a progress report on the latest developments on the European scene in this field. There is not any change. The only statistically valid example of immunotherapy successfully maintaining remissions in acute leukemia remains that of Doctor Mathé. He and his colleagues had attempted a similar trial in Britain which failed. They studied leukemic patients, all of whom were in remission, who had been given chemotherapy for a finite period of time. BCG alone appeared to be ineffective in preventing relapse, as compared with the control groups. However, there were several points of difference between the British study and the study of Mathé. The British group used Glaxo BCG, whereas Mathé used BCG prepared at the Pasteur Institute, and there are physiologic if not physical differences between these preparations. Secondly, because they did not have stored leukemic cells, they could not give specific immunization at the time and, therefore, they were giving only nonspecific stimulations. Since then, much work has shown that the effect of nonspecific stimulation of the reticuloendothelial system is trivial in terms of immunotherapy. In fact, one would never choose to do the study that way again. It was because at the time they did not have access to a sufficient number of irradiated leukemic cells. Third, although Mathé's 3 groups received either BCG alone or irradiated cells alone or both, as soon as he became convinced that both was better than either alone, all the patients got both so that he does not have a pure BCG group either.

However, the British group now have the possibility of using irradiated leukemic cells because they have an IBM machine which can separate them off very nicely. In this context, some of the in vitro results obtained at the Royal Marsden Hospital in which patients were immunized with their own irradiated tumor cells are relevant. In the mixed-lymphocyte reaction, if one takes leukemic cells and stores them, one can then bring the patient into remission and take the patients' lymphocytes. Those lymphocytes will react against the previously stored leukemic cells as if they were foreign. It seemed of interest to learn in what way the reaction of the patients' lymphocytes against the leukemic cells might be altered after immunization. They measured the change in isotope uptake in the mixed-lymphocyte reaction when patients were stimulated by being immunized by their own autologous leukemic cells. Sometimes there was a very marked increase in the ability of the patient's lymphocytes to recognize his own leukemic cells as foreign, though this response tended to be very transitory. In one patient immunized with cells and BCG, the reaction fluctuated considerably. In another patient, there was a considerable difference between the reaction of his lymphocytes against his leukemia cells in fetal calf serum compared with the activity of his lymphocytes against his own blast cells in his own fresh serum. In other words, there appeared to be a serum factor inhibiting the mixed-lymphocyte reaction which does not occur in fetal calf serum. The situation can in fact be quite complicated. On some occasions in the serum there would appear to be inhibiting factors and on other occasions there would appear to be stimulatory factors. What is the nature of these factors? What is their relationship to the timing of immunization? These points remain to be worked out.

One great difficulty about Hodgkin's disease has been that one has not had an antigen which one could identify and against which one could measure immunologic responses. Now that we see the glimmerings of this possibility, it seems likely that a great deal more will be learned about the immunology of Hodgkin's disease. But whatever is learned, two things will be apparent. In order for immunotherapy to be used in Hodgkin's disease, we will have to have either a patient with an intact immune system or one who, as a result of being stimulated, gains an intact system. Secondly, we will need to have some suitable preparation of specific antigen with which to stimulate him.

Kaplan referred to the experiments by Doctor Latarjet in Paris who immunized patients with Hodgkin's disease with their own heavily irradiated tissue. He asked whether any of the participants knew what results were being obtained. He also asked what the prospects might be for the use of hapten-conjugated tumor tissue to immunize. **M. Tubiana** replied that, to the best of his knowledge, Doctor Latarjet did not yet have any conclusive results. **Fairley** commented that treating malignant cells with neuraminidase has made them very much more antigenic in a number of systems. Unfortunately though, this does not happen with all animal tumors, so one cannot argue that it is a general biologic phenomenon. Secondly, it would be prohibitively costly as a clinical procedure.

Carbone asked about the current technique of BCG treatment being used in leukemic patients in Britain. **Fairley** replied that they are not giving the BCG in the same way as Doctor Mathé, who mixes BCG with the leukemic cells. It is important to clear up one point about British versus French BCG. The British preparation has been said to be ineffective and, of course, in this context it was, but it is quite effective in animal experiments. His group has compared it in animal experiments with the French BCG made by the Pasteur Institute, and obtained comparable responses. There was also a study in which the British BCG was compared in vivo in man but there, unfortunately, it was not injected in the same way. At the moment they are using either autologous or allogeneic cells and BCG in acute monoblastic leukemia as an adjuvant treatment with chemotherapy. They are not relying on it alone. They have a chemotherapy control group and a chemotherapy plus immunotherapy group. **Hersh** asked whether Doctor Fairley was concerned about the possibility that concurrent chemotherapy causes enough immunosuppression to increase the risk of BCG dissemination. **Fairley** replied that he was very concerned, but had never seen it. The session was then adjourned.

SESSION 6

SURVIVAL DATA AND PROGNOSIS

Chairman: Byron W. Brown, Jr.

INTRODUCTION

As a biostatistician, I am often approached by my medical colleagues for advice. Sometimes they have a minor pain in their standard deviation and I can treat them, send them home and tell them to come back if it gets worse, but when somebody tells me that he is interested in Hodgkin's disease, I know that we have a Stage IV case. I can tell you some of the symptoms: low patient accrual rates, very long-term follow-up, a confusing wealth of baseline data, some of which are used in staging and some of which are to be used only at the end of the study, adjustment of X-ray positives for differences in baseline variables in several treatment groups, varying lengths of observation causing the censoring of data and requiring such exotic statistical treatment as the use of actuarial survival methods, and a variety of endpoints associated with relapse and death, each presenting problems of definition (even death causes problems as to what is its cause), and puzzles in regard to their interrelationships and advantages in the use and evaluation of data. We will be hearing reports on many of these concerns and on some of the current and new methods used in handling these problems.

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Survival and Relapse Rates in Hodgkin's Disease: Stanford Experience, 1961–71 [1,2]

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SUMMARY—Actuarial analyses of survival and freedom from relapse are presented for a coherent series of 613 biopsy-proved, previously untreated patients with Hodgkin's disease admitted to Stanford University Medical Center from 1961–71. All cases were staged according to the Ann Arbor clinical classification after routine lymphangiography; staging laparotomies were also performed in many cases. The evolution of treatment policies during the decade is described. Overall survival was 75.9% at 5 years, corrected for intercurrent deaths. The major factors affecting prognosis were clinical stage, constitutional symptoms, histopathologic type, and age >40 years; females had only a slightly better prognosis than males, probably ascribable mainly to differences in stage distribution and histopathologic type.—Natl Cancer Inst Monogr 36: 487–496, 1973.

THE PAST DECADE has witnessed the introduction of a number of major advances in the diagnostic evaluation, staging, and treatment of patients with Hodgkin's disease. Among these advances are the use of lymphangiography for the detection of occult involvement in the para-aortic and pelvic lymph nodes (*1, 2*), the use of staging laparotomy for the detection of spleen and liver involvement, as well as for biopsy confirmation of para-aortic node disease (*3*), and the more rigorous staging of cases in accordance with modern classifications such as that adopted at Ann Arbor in 1971 (*4*). Meanwhile, the advent of cobalt-60 teletherapy apparatus, linear electron accelerators, and betatrons ushered in the megavoltage era of radiotherapy and enabled radiotherapists to use large fields shaped to encompass multiple lymph-node chains and total doses in the tumoricidal range for the treatment of the malignant lymphomas, including Hodgkin's disease (*5*). Finally, during the past decade, single-drug palliative chemotherapy gave way to combination multidrug chemotherapy, which appears to have significantly increased both the probability of induction of complete remissions and the duration of remission in the unmaintained state (*6*).

It seemed desirable to examine survival data in a large series of previously untreated, biopsy-proved cases of Hodgkin's disease, all of which had been staged with the aid of lymphangiography and many also with the aid of staging laparotomy at Stanford University Medical Center from 1961 through December 31, 1971. This paper presents the results of such an analysis.

MATERIALS AND METHODS

All previously untreated patients admitted to and treated at Stanford University Medical

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[3] I gratefully acknowledge the expert assistance of Mr. Vincent Parziale and Mrs. Polly Butterfield with the computer input, programming, and analysis of the data.

Center between 1961, when routine lymphangiography was first introduced, and December 31, 1971, comprise the material for this analysis. Patients with all stages of disease, including those with Stage IV disease who received only palliative chemotherapy or palliative radiotherapy, are encompassed. Parameters examined included age, sex, clinical stage, the presence or absence of constitutional symptoms, and histologic type. The Ann Arbor clinical staging classification (*4*) was applied retrospectively to all cases. Cases with localized extralymphatic sites of involvement, which were formerly classified in Rye Stage IV, were reallocated to Stages II_E or III_E, in accord with the criteria defined in the Ann Arbor staging classification. Except for a small residue of about 75 unclassified cases during the period 1967 to June 1968, all were classified with respect to histopathologic type, with the Rye classification (*7*) of lymphocytic predominance (LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocytic depletion (LD). Cases classified before 1967 were included in the paper by Keller et al. (*8*) and reviewed by 3 pathologists. Those reviewed prospectively from July 1968 through 1971 were interpreted and classified by Dr. Ronald F. Dorfman, Associate Professor of Pathology and Codirector, Laboratory of Surgical Pathology, Stanford University Medical Center. Survival and relapse-free survival were analyzed actuarially by the method of Berkson and Gage (*9*), with and without correction for intercurrent deaths, with an IBM 360-50 computer. All curves were terminated when <5 cases remained at risk in any given group.

Treatment policies at Stanford University Medical Center in the management of patients with previously untreated Hodgkin's disease evolved considerably in the decade under study. Specifically, 2 randomized clinical trials were initiated in May 1962, and a series of other trials were initiated at subsequent times during the decade, the designs of which were fully described in (*10–13*). Criteria for eligibility in these protocol studies were carefully defined, and patients who were excluded from the trials because they did not meet the criteria for eligibility were treated in accordance with our best judgment at the time. Both protocol and nonprotocol cases are included in the present analysis.

Specifically, patients with Stage I and Stage II disease were treated with curative intent throughout the decade, with high-dose megavoltage radiotherapy, usually to tumor doses of 4400 rads in 4 weeks. However, the extent of the treatment fields was the subject of investigation in one of the clinical trials, and half the protocol cases of Stage I and Stage II disease initially were randomized to high-dose treatment limited to the involved chains of lymph nodes (involved-field or IF technique), whereas the other half were treated through fields extended to encompass adjacent, apparently uninvolved lymph-node chains as well (extended-field or EF technique). After July, 1967, the fields were further extended to encompass all major lymph-node chains, both above and below the diaphragm (total nodal or TN technique). In the earlier years of the decade, nonprotocol cases were treated primarily by the IF or EF techniques; but, from 1967 on, essentially all Stage I and II nonprotocol cases have been treated by the TN technique.

In Stage III, treatment at the beginning of the decade was universally palliative because cases with Stage III extent were then regarded as incurable. However, in May 1962, a randomized clinical trial was initiated to test the hypothesis that treatment with curative intent might be able to salvage a significant fraction of cases of Stage III Hodgkin's disease. Accordingly, from that date through 1965, half the protocol cases were allocated at random to low-dose palliative radiotherapy to involved chains only, whereas the other half were allocated to TN high-dose radiotherapy, as described in (*10, 11*). Nonprotocol cases, beginning in 1965, and all protocol cases from that time on, when the highly favorable results of treatment with curative intent began to be apparent, were treated by the TN technique.

In 1968, the policy was changed further with a new randomized, clinical trial: Half the cases of Stage III-A and Stage III-B disease were allocated to TN radiotherapy alone, whereas the other half were allocated at random to TN radiotherapy followed, after a rest period of several weeks, by 6 cycles of MOPP (a combination of mechlorethamine, vincristine, procarbazine, and prednisone) quadruple drug chemotherapy. Preliminary results have been favorable (*13*). This experience in turn has influenced treatment policy in Stage IV, which was uniformly palliative in approach through 1968. At that time, most cases were treated with single-drug chemotherapy, sometimes supplemented by low-dose radiotherapy. One exception to this approach was in a small group of patients, classified as Stage IV by virtue of documented liver involvement, who were successfully treated experimentally with TN radiotherapy followed by intravenous colloidal radioactive gold (^{198}Au) (*14, 15*). Except for this small group of Stage IV cases, all others during that time were treated palliatively. However, beginning in 1968, our chemotherapeutic treatment policy shifted from the traditional single-drug method to the use of MOPP quadruple drug chemotherapy (*6*), for $\geqq$6 cycles, followed by less intensive maintenance chemotherapy with a variety of regimens. Finally, since 1970, because of our encouraging results in Stage III-B disease treated with both radiotherapy and MOPP (*13*), some cases with Stage IV disease were randomized to "split-course" treatment; they received 2 cycles of MOPP quadruple drug therapy, then TN radiotherapy, and finally the remaining 4 cycles of MOPP chemotherapy and low-intensity maintenance chemotherapy.

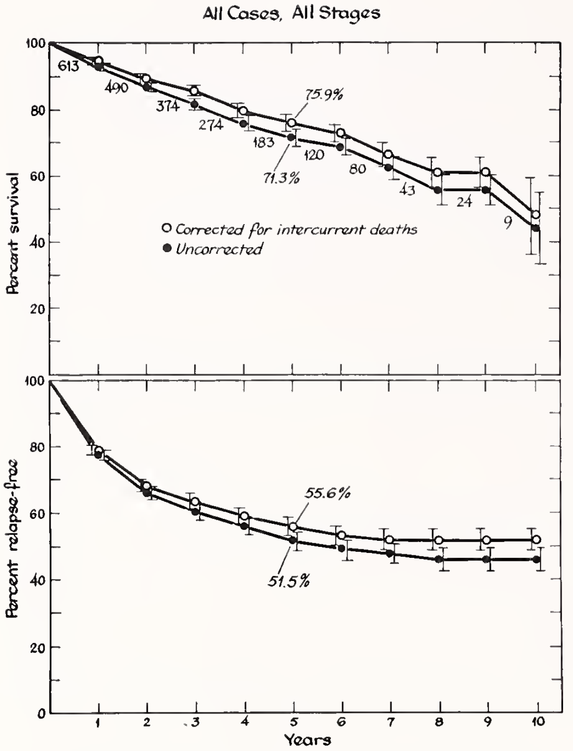
The results of these varying treatment techniques will, of course, ultimately become apparent in the various clinical trials and will be reported separately. In the present study, the composite effects of both the more intensive and more limited treatment policies are necessarily intermingled in an inxerticable fasion. In general, all Stage I and Stage II cases were treated with curative intent, but with the extent of treatment fields changing in the direction of increasing comprehensiveness during the decade. Stage III cases were, in part, treated palliatively at the beginning of the decade and were more uniformly accepted for treatment with curative intent only during the last half of the decade. Finally, Stage IV cases were treated palliatively during almost the entire decade, with a small number receiving radical treatment for documented involvement of the liver as described and an additional small number receiving MOPP quadruple drug chemotherapy, with or without supplemental TN radiotherapy, in the last 2 years of the decade under consideration.

OVERALL SURVIVAL DATA

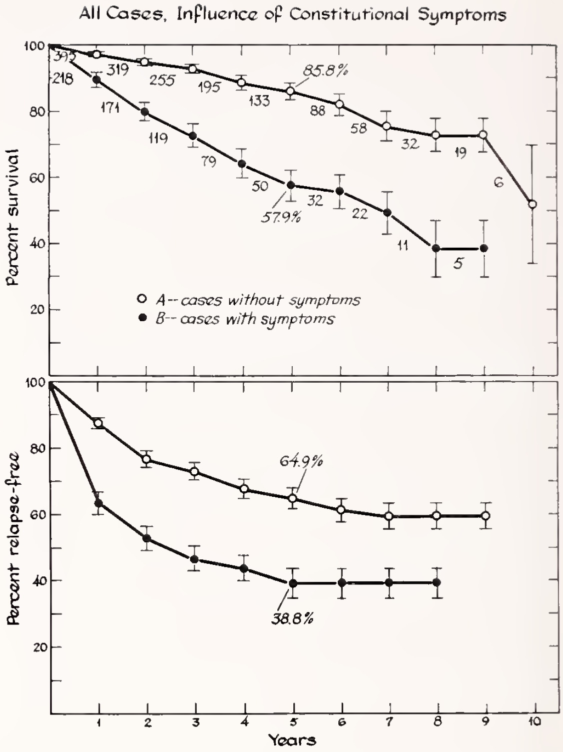
The actuarial survival of the total series of 613 cases is presented in text-figure 1. The number of cases at risk during each annual interval is indicated in the space between data points, and the standard error of each survival value is indicated with small bars. Survival data are presented both in uncorrected form and with corrections for intercurrent deaths, unrelated to Hodgkin's disease, occurring during each annual interval. As might be expected with a relatively young patient population, the difference between the 2 survival curves was relatively small. Five-year survival for the entire series was 71.3% uncorrected and 75.9% corrected. Survival continued to fall off during the second 5-year period, reaching a level of 45–50% for the entire series at the 10th year. It is likely that this reflects, in part, the more limited treatment techniques used in the first part of the decade under study. These survival data were essentially similar to those previously reported from this institution for smaller numbers of cases (*12, 16, 17*) and, as has been pointed out previously, represent a dramatic improvement over representative survival data obtained for Hodgkin's disease during the kilovoltage era of radiotherapy (*12, 17*).

FREEDOM FROM RELAPSE

The lower panel of text-figure 1 presents the actuarial analysis of the proportion of cases remaining relapse-free in each annual interval during the decade. The greatest incidence of relapse occurred during the first 3 years, in keeping with our previous experience (*5, 18*). The curve then flattened out by the 7th year and remained unchanged thereafter through the 10th year at approximately 52%. It is therefore reasonable to assume that these relapse-free cases represent a minimal estimate of the total proportion of cases in the entire series that will achieve permanent cure. It is likely, however, that a substantial number of additional patients



TEXT-FIGURE 1.—Actuarial analysis of survival and freedom from relapse, with and without correction for intercurrent deaths, in a series of 613 cases of all stages of Hodgkin's disease, 1961–71.



TEXT-FIGURE 2.—Actuarial analysis of survival and freedom from relapse, for patients without (A) and with (B) constitutional symptoms: unexplained fever, night sweats, and/or loss of more than 10% of normal body weight.

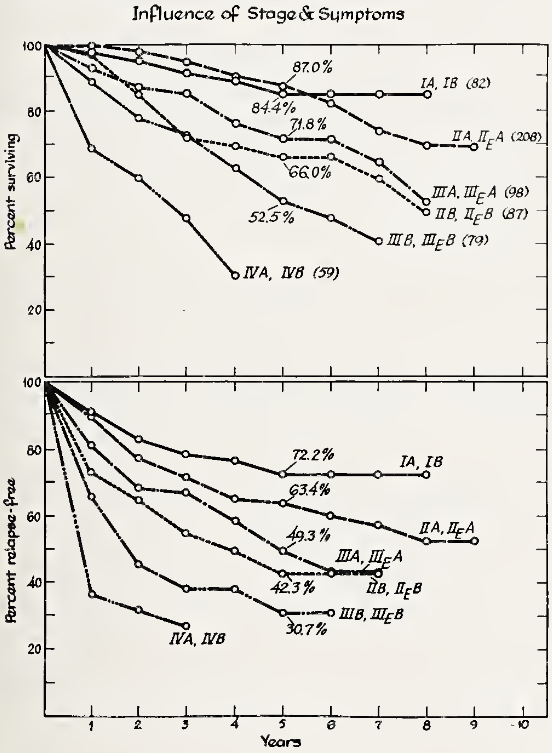
who suffered relapses were successfully salvaged by supplementary radiotherapy and/or combination chemotherapy. Thus the total cure rate may be expected to exceed the relapse-free baseline.

INFLUENCE OF CONSTITUTIONAL SYMPTOMS

Analysis of the entire series of 613 patients reveals 395 who had no significant constitutional symptoms and 218 who had unexplained fever, night sweats, and/or weight loss exceeding 10% of normal body weight at the time of admission. These 2 groups were analyzed separately for actuarial survival and freedom from relapse, corrected for intercurrent deaths (text-fig. 2). Once again, the adverse impact of constitutional symptoms on prognosis in Hodgkin's disease is evident in both sets of curves. Five-year survival for the asymptomatic (A) group was 85.8%, whereas that for the symptomatic (B) group was 57.9%. The A group had a flattening out of the relapse-free survival curve at approximately 59% in the 8th year, with no change thereafter through the 10th year, whereas the B group fell to approximately 38% as early as the 5th year and stabilized at that level thereafter. Thus there was an approximately 20% difference in prognosis which, for patients with disease of all stages, was apparently attributable to the presence or absence of constitutional symptoms.

INFLUENCE OF CLINICAL STAGE

There were so few cases in Stage I-B and in Stage IV-A that these 2 small groups were added to those of Stages I-A and IV-B, respectively, for this analysis. In Stages II and III, however, the A and B groups were analyzed separately. Patients with localized extralymphatic (E) sites of involvement were previously found to have the same prognosis as those with lymphatic disease of corresponding extent, in keeping with the observations of Musshoff and Boutis (*19*) and of Peters et al. (*20*). Therefore they were grouped with the cases having a corresponding extent of lymphatic involvement. Thus Stage II$_E$-A cases were grouped with Stage II-A cases, and Stage II$_E$-B cases with Stage II-B, etc. The total numbers of cases at risk in each group are indicated in parentheses in text-figure 3, which presents the data for both survival and freedom from relapse for the various stages.



TEXT-FIGURE 3.—Actuarial analysis of survival and freedom from relapse for the entire series, subclassified by stage according to the Ann Arbor clinical staging classification (*4*).

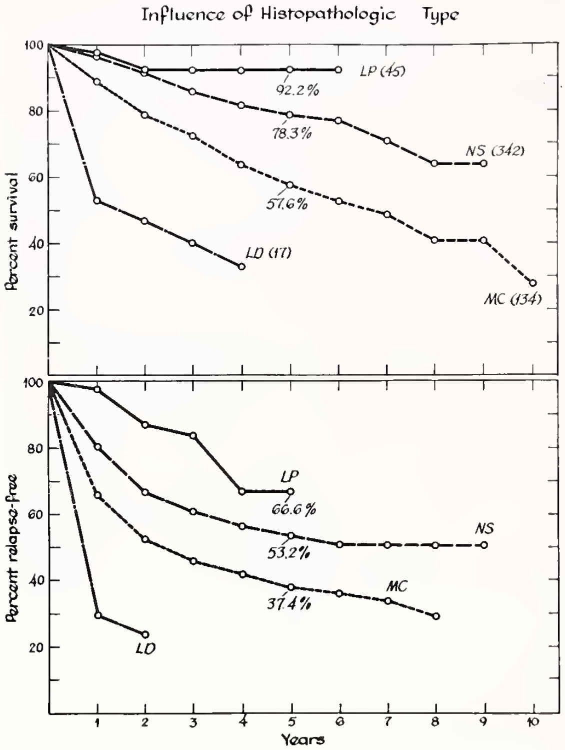
The prognostic discriminatory power of the 4 Ann Arbor clinical stages and their subclassification into A and B subgroups is again validated in these data. Survival in Stage I flattened out after the 5th year at approximately 84%, and 72% of these cases remained relapse-free after the 5th year. In Stages II-A and II$_E$-A, survival during the first 5 years was actually slightly better than that in Stage I, but there was a significant number of deaths during the second 5-year period, with the result that survival by the 9th and 10th years had fallen to approximately 69%, of which some 52% remained relapse-free. Perhaps most interesting of all are the data on Stages III-A and III$_E$-A (which at the beginning of this decade would have been universally regarded as incurable) in which 5-year survival exceeded 70%, and some 42% remained relapse-free into the 7th year.

The adverse prognostic influence of constitutional symptoms is again evident in the curves for Stages II-B and II$_E$-B, as well as in those for III-B and III$_E$-B, which were significantly poorer than those for the corresponding asymptomatic groups. Nonetheless, it is significant that about 42% of those in Stage II-B and II$_E$-B and about 31% of those in Stage III-B and III$_E$-B remained relapse-free beyond the 5th year. Finally, the data for Stage IV, as might have been expected, were the most unfavorable of all, both with respect to survival and freedom from relapse.

INFLUENCE OF HISTOPATHOLOGIC TYPE

The data of text-figure 4 once again testify to the prognostic discriminatory value of the Rye histopathologic classification, as previously reported by several groups of investigators (*8, 21–23*). Thus survival in patients with LP histology flattened out at approximately 92% after the 2d year, with about two-thirds of these cases remaining relapse-free into the 5th year. The NS form of Hodgkin's disease was also relatively favorable, with a 5-year survival of about 78%, 10-year survival of about 63%, and a relapse-free survival rate of approximately 50% from the 6th through the 9th year. The MC form was distinctly more unfavorable, with 5-year survival of 57%, 10-year survival of <30%, and slightly <30% remaining relapse-free in the 8th year. Moreover, there was no indication of a stabilization of the relapse-free survival curve in this histologic category, which suggests that some additional late relapses may be observed beyond the 8th year, in striking contrast to the LP and NS categories. Finally, as expected, the

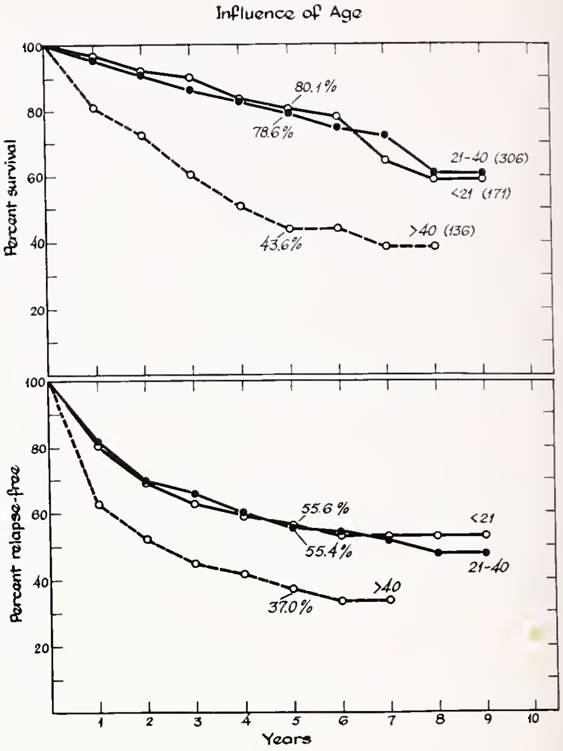
LD group, though too small to be statistically valid, had the poorest prognosis both in terms of survival and freedom from relapse.



Text-figure 4.—Actuarial analysis of survival and freedom from relapse according to histopathologic type, with the Rye classification (*7*).

INFLUENCE OF AGE

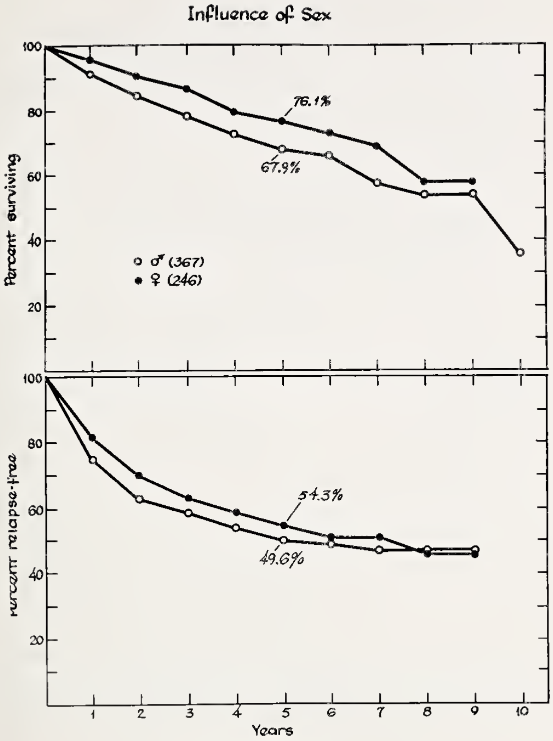
Survival and relapse data were analyzed separately for 3 different age groups: <21 years (171 patients), 21–40 years (306 patients), and ≧41 years (136 patients). The data presented in text-figure 5 reveal no appreciable difference between the young-adult group and the pediatric and adolescent age group. However, prognosis was distinctly more unfavorable for patients >40 years old, a confirmation of previous observations (*17*). When the data for the youngest age group were examined more closely, there were no indication of an adverse prognosis for those patients <12 years old and no support whatever for the widely prevalent impression that children with Hodgkin's disease have an unfavorable prognosis.



Text-figure 5.—Actuarial analysis of survival and freedom from relapse in 3 different age groups.

INFLUENCE OF SEX

Survival for the 367 male patients in the series was approximately 68% at the 5th year, but fell to 36% by the 10th year (text-fig. 6). Survival for the 246 female patients was about 10% higher than that for the males during the first 5-year period, with a slightly smaller difference by the 9th year. However, the relapse data showed a much smaller difference, which disappeared entirely by the 8th year. Because of the fact that the female patients tended to have a somewhat more favorable clinical stage distribution and a greater prevalence of NS histology than the males (*17*), the small difference in prognosis favoring the females is probably attributable to these factors rather than to any inherently greater resistance or slower rate of evolution of Hodgkin's disease among female than among male patients.



TEXT-FIGURE 6.—Actuarial analysis of survival and freedom from relapse for males versus females.

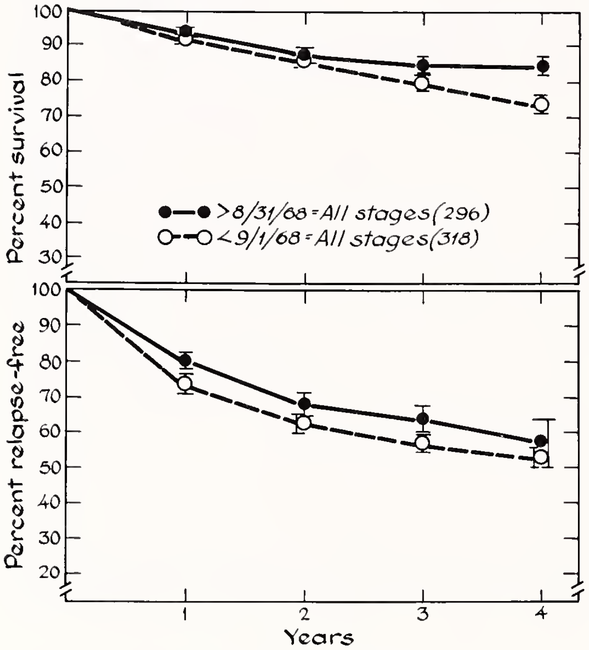
INFLUENCE OF STAGING LAPAROTOMY AND/OR COMBINATION CHEMOTHERAPY

When the value of staging laparotomy and splenectomy in selected patients with Hodgkin's disease became apparent (*3*), it was decided to adopt this procedure *routinely* as part of the diagnostic evaluation of all but clinically overt Stage IV cases, effective September 1, 1968. More or less concurrently, our preliminary experience with MOPP quadruple drug chemotherapy in selected relapsing and Stage IV cases had proved favorable enough to initiate new protocols which included MOPP, as a supplement to radiotherapy, as one of the treatment options. Although admittedly other variables could also have changed in the ensuing 4 years, the only other change we observed was the occurrence of a greater proportion of Stage III and IV cases (table 1), due in part, undoubtedly, to the reallocation of cases after staging laparotomy but in part also to changing referral patterns. Accordingly, it seemed of interest to compare our data for survival and freedom from relapse in the prelaparotomy and postlaparotomy eras, with September 1, 1968, as the dividing line. If staging laparotomy and MOPP were to favorably influence prognosis, they would do so primarily in the more advanced stages (III and IV) of the disease. If such an improvement were due merely to reassortment of cases from one stage to another, however, there should be no favorable influence on the overall prognosis.

TABLE 1.—*Stage distribution of cases*

Stage (Ann Arbor)	Date of admission			
	Before 9/1/68		After 8/31/68	
	Number	Percent	Number	Percent
I	45	14.3	37	12.5
II	164	51.6	131	44.3
III	85	26.7	93	31.4
IV	24	7.5	35	11.8
Total	318		296	

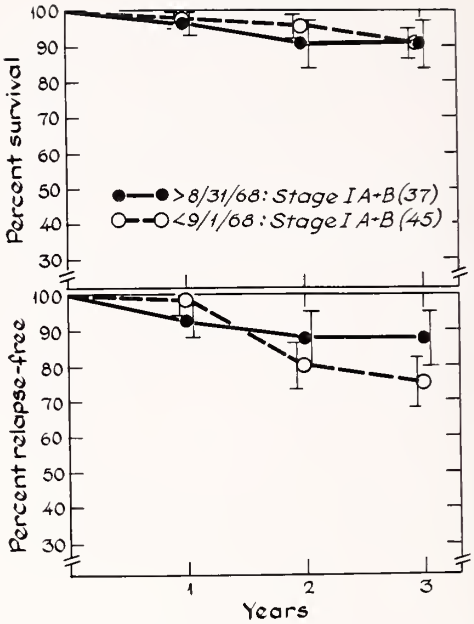
Actuarial survival and relapse-free survival data for all (previously untreated) cases of all stages admitted between 1961 and August 31, 1968, ("<9/1/68") are compared in text-figure 7 with those for all (previously untreated) cases admitted from September 1, 1968, through December 31, 1971 (">8/31/68"). The corresponding comparisons by stages are presented in text-figures 8–11. Text-figure 7 shows that overall survival indeed improved at 4 years, from 73.7% in the earlier group to 84.9% in the more recent group, and that the rates for freedom from relapse ran parallel and about 5–6% higher for the recent group. As expected, there was no difference in survival (at 3 yr) between the 2 groups of Stage I cases (text-fig. 8). However, survival in Stage II apparently differed appreciably (81.8% vs. 92.9% at 4 yr; text-fig. 9). An appreciable difference in both survival (65.8% vs. 81.9%) and in freedom from relapse (42.6% vs. 58.0%) was noted at 4 years in the Stage III cases (text-fig. 10). Finally, the greatest differences were seen in Stage IV (text-fig. 11), where survival at 3 years had improved from 33.3–61.7%, and freedom from relapse, which had fallen to 16.7% in the earlier group, had climbed to 44.1% in the recent group. It would obviously not be appropriate to ascribe these differences exclusively to staging laparotomy, MOPP chemotherapy, or the combination of the two. Nonetheless, it seems highly probable that they are, at least in part, responsible for these encouraging trends in prognosis.



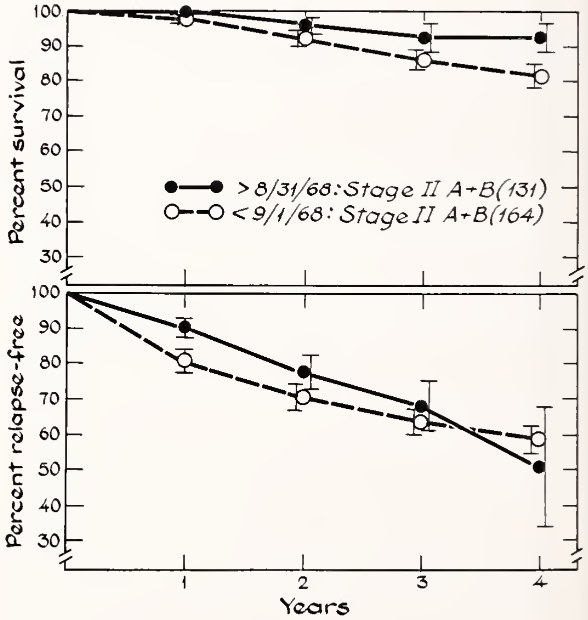
TEXT-FIGURE 7.—Actuarial analysis of survival and freedom from relapse in 318 patients with all stages admitted between 1961 and August 31, 1968 ("<9/1/68") versus 296 patients admitted from September 1, 1968, through December 31, 1971 (">8/31/68"). The more recent group was routinely evaluated with the aid of staging laparotomy and splenectomy, and MOPP combination chemotherapy was used in the treatment of many patients with Stage II-B, III-A and -B, and IV disease.

DISCUSSION

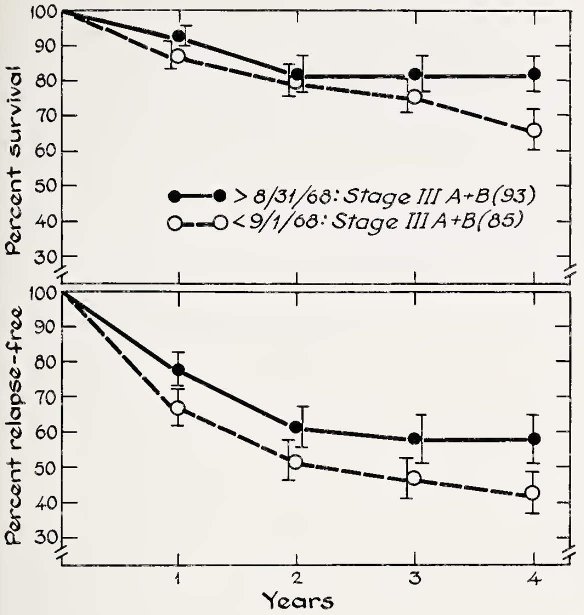
The overall data once again testify to the dramatic improvement in the prognosis of Hodgkin's disease that was achieved in the past decade, primarily because of better diagnostic evaluation and improved radiotherapy and chemotherapy (*17*). Nonetheless, much of the improvement is attributable to the results achieved in relatively favorable situations (clinical Stages I, II, and III-A, and LP and NS histologic types). There is still a great deal of room for improvement evident in the much more unfavorable survival and relapse experience of



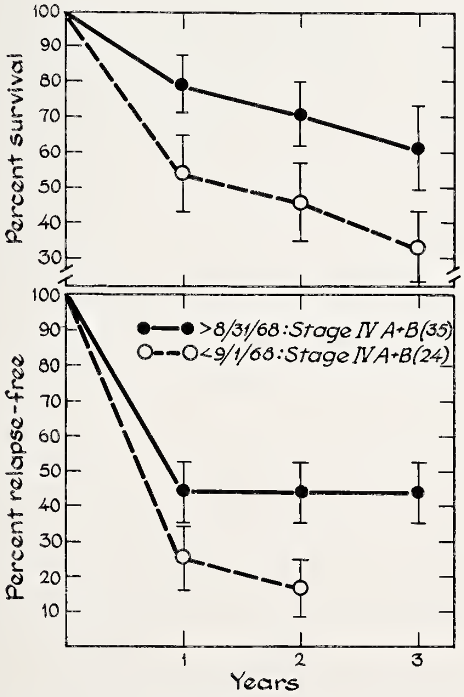
TEXT-FIGURE 8.—Actuarial analysis of survival and freedom from relapse for the Stage I cases among the 2 groups described in the legend to text-figure 7.



TEXT-FIGURE 9.—Actuarial analysis of survival and freedom from relapse for the Stage II cases among the 2 groups described in the legend to text-figure 7.



Text-figure 10.—Actuarial analysis of survival and freedom from relapse for the Stage III cases among the 2 groups described in the legend to text-figure 7.



Text-figure 11.—Actuarial analysis of survival and freedom from relapse for the Stage IV cases among the 2 groups described in the legend to text-figure 7.

patients with Stage II-B, III-B and IV disease, as well as in those with the prognostically unfavorable MC and LD histopathologic types. Hopefully, further advances in the use of total-lymphoid radiotherapy in conjunction with combination chemotherapy (*13*) during the next decade will significantly improve the prognosis of these unfavorable groups and thus further improve the overall prognosis of Hodgkin's disease.

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Analysis of Primary Relapses After Radiotherapy in Hodgkin's Disease [1,2]

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SUMMARY—This is a detailed retrospective analysis of 114 relapses among 426 patients with previously untreated, biopsy-proved Hodgkin's disease, Stages I-III, admitted between 1961 and December 31, 1970. The parameters studied included age, sex, clinical stage, histologic type, interval to first relapse, interval to second relapse, site of relapse, type of radiotherapy, and prognosis. The unfavorable influence of a primary relapse on prognosis was again documented; transdiaphragmatic and extralymphatic relapses were significantly more difficult to control than local or regional relapses.—Natl Cancer Inst Monogr 36: 497–508, 1973.

PREVIOUS STUDIES clearly demonstrated that the interval between initial radiotherapy and the occurrence of a primary relapse is an important factor in prognosis and that the occurrence of an initial relapse adversely affects prognosis to a very significant extent (*1–4*). It was also established that the occurrence of a primary relapse in a radiotherapeutically treated field (defined as a "true recurrence") is an inverse function of dose; the true recurrence rate decreases sharply as radiation dose increases, down to a level of 1.3% at a total dose level of 4400 rads delivered in 4 weeks (*5, 6*). However, there has been no previous comprehensive analysis of the occurrence of primary relapses of various anatomic distributions, relative to age, sex, clinical stage, constitutional symptoms, histopathologic type, or technique of treatment in previously untreated patients with Hodgkin's disease. This paper presents such an analysis, based on retrospective studies of a large series of patients admitted to Stanford University Medical Center between 1961 and December 31, 1970.

Inasmuch as the study was to analyze relapses among patients treated with curative intent by radiotherapy, all patients receiving only palliative treatment, either radiotherapeutic or chemotherapeutic, as primary treatment for Stage III or Stage IV disease were excluded. This left a net total of 462 patients with previously untreated, biopsy-proved Hodgkin's disease admitted between 1961 and December 31, 1970, all of whom had been staged with the aid of lymphangiography and many, in addition, with the aid of staging laparotomy (7). Among these patients, a total of 114 (24.7%) had developed a primary relapse before December 31, 1970.

All patients were retrospectively restaged in accordance with the recent Ann Arbor clinical staging classification (*8*), whereby patients with

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localized extralymphatic sites of involvement are reallocated to those stages determined by the associated extent of lymphatic disease and then denoted additionally with the suffix "E." Accordingly, all patients who were formerly in Rye Stage IV and who had such localized extralymphatic disease have been included in the present analysis and are designated as Stage II_E-A or B or Stage III_E-A or B, depending on the extent of associated lymphatic involvement. Those remaining in Ann Arbor Stage IV, by virtue of the presence of disseminated or diffuse disease in tissues, such as the liver, bone marrow, both lungs, or multiple, widely scattered osseous or subcutaneous sites, have not been included in this analysis, primarily because most of them received merely palliative treatment.

All the patients before 1966 were reviewed and classified retrospectively in accordance with the Rye histopathologic classification (*9*) by Keller et al. (*10*); all patients admitted since July 1, 1968, were reviewed and classified in accordance with the same histopathologic classification by Dr. Ronald F. Dorfman, Associate Professor of Pathology and Co-director, Laboratory of Surgical Pathology, Stanford University Medical Center. Approximately 75 patients admitted between 1966 and mid-1968 have remained unclassified, but all of them suffering primary relapses were reviewed and retrospectively classified by Dr. Dorfman. The abbreviations used in the paper are as follows: LP for lymphocytic predominance; NS for nodular sclerosis; MC for mixed cellularity; and LD for lymphocytic depletion.

In accordance with a previous proposal (*5*), the terms "relapse," "recurrence," and "extension of disease" are not used interchangeably; instead, "relapse" refers to *any* new manifestation of disease after the initial course of radiotherapy, "recurrence" to the appearance of a primary relapse in a previously irradiated field, and "extension" to a primary relapse in an area not previously irradiated. Relapses have been further subclassified in this paper in accordance with a proposal made at this Symposium, which suggested that all relapses be categorized as follows:

1. *Local.* Any primary relapse appearing in a previously involved chain of lymph nodes within a treated field.
2. *Regional.* Any primary relapse appearing on the same side of the diaphragm as initially treated disease, including marginal recurrences.
3. *Transdiaphragmatic.* Any primary relapse occurring in the lymphatic system, including the spleen, on the opposite side of the diaphragm from initially treated disease.
4. *Extralymphatic.* Any primary relapse appearing initially in an extralymphatic tissue or organ and further subclassified as follows: *a*) liver; *b*) bone marrow; *c*) lung; and *d*) other (subcutaneous, bone, central nervous system, etc.).

The type of radiotherapy which had been used in the treatment of the relapsing cases was classified for the purposes of this analysis as follows:

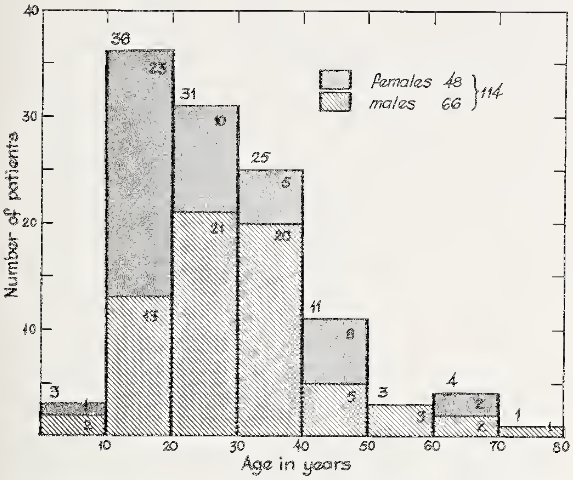
1. *Involved-field* (*IF*). Local radiotherapy encompassing only chains of lymph nodes known to be involved with disease, usually carried to a dose of 4400 rads in 4 weeks.
2. *Extended-field* (*EF*). Treatment encompassing 1 or more apparently uninvolved lymph-node chains beyond those known to be involved with disease; sometimes this included all chains above the diaphragm and the para-aortic chain, but not the pelvic lymph nodes. All treated areas were usually carried to the same total dose level (4400 rads/4 wks).
3. *Total-nodal* (*TN*). Treatment to all major lymph-node chains, from the upper cervical level down to and including the inguinal and femoral lymph nodes. In the earlier part of this study, 10 patients receiving TN treatment did not receive treatment to the spleen (or the splenic pedicle in splenectomized cases). All other patients included in the TN group received spleen and/or splenic pedicle irradiation in addition to treatment of all lymph-node chains. The dose to all involved sites was at least 4400 rads, and that to all apparently uninvolved sites in the TN group at least 3500 rads.

The X-ray beams of 2 linear accelerators, operating at 5 and 6 Mev, respectively, were used at a working distance of 120 cm or more in the treatment of all patients, with techniques fully described elsewhere (*11–13*).

RESULTS

Composition of Relapsing Cases

The age and sex composition of the series of 114 patients with primary relapses is presented in text-figure 1. There were a total of 66 males and 48 females, for a sex ratio of 1.4. It is interesting that 23 of the 48 females, or nearly 50%, were in the 11- to 20-year age bracket. In contrast, the males were much more widely and homogeneously distributed, with a broad peak from 11 through 39 years of age.



TEXT-FIGURE 1.—Age and sex composition of patients with primary relapses.

The distribution of relapsing cases by Ann Arbor clinical stage and treatment technique is given in table 1. A total of 14 patients were in Stage I, 39 in Stage II-A, 25 in Stage II-B, 14 in Stage III-A, and 22 in Stage III-B. Radiotherapy was given to 28 patients by the IF technique, to 39 patients by the EF technique, and to 47 patients by the TN technique. The fact that the TN technique was used selectively for patients with Stage III disease accounts for the much greater proportion of Stage III-A and Stage III-B relapses occurring in the TN group.

TABLE 1.—*Distribution of relapsing cases by clinical stage and treatment technique*

Clinical stage (Ann Arbor, 1971)	Radiotherapy technique			
	IF	EF	TN	Total
I-A	8	5	0	13
I-B	0	1	0	1
II-A	10	24	5	39
II-B	7	7	11	25
III-A	2	1	11	14
III-B	1	1	20	22
Total	28	39	47	114

Risk of Relapse

The relative risk of developing a primary relapse after radiotherapy of Hodgkin's disease was analyzed in relation to a number of relevant factors. The influence of both histologic type and clinical stage is presented in table 2. Of the total series of 462 patients, there were 64 patients with diseases classified in Stage I-A, of whom a total of 13 (20%) developed primary relapses. The corresponding data for the other stages were as follows: Stage I-B, 1 of 2 (50%); Stages II-A and II$_E$-A, 39 of 164 (24%); Stages II-B and II$_E$-B, 25 of 93 (27%); Stages III-A, III$_E$-A, and III$_S$-A, 14 of 71 (20%); and Stages III-B, III$_E$-B, and III$_S$-B, 22 of 68 (32%). There were only 6 relapses among the 49 pa-

TABLE 2.—*Distribution of primary relapses by stage and histologic type among 462 cases of Hodgkin's disease, Stages I-III*

Clinical stage*	Histologic type†				Total
	LP	NS	MC	LD	
I-A	3/19 (16)	5/26 (19)	5/17 (29)	0/2 (0)	13/64 (20)‡
I-B	—	1/2	—	—	1/2
II-A, II$_E$-A	3/21 (14)	29/110 (26)	6/32 (19)	1/1	39/164 (24)
II-B, II$_E$-B	0/3 (0)	15/66 (23)	8/22 (36)	2/2	25/93 (27)
III-A, III$_E$-A, III$_S$-A	0/6 (0)	9/41 (22)	5/24 (21)	—	14/71 (20)
III-B, III$_E$-B, III$_S$-B	—	9/32 (28)	9/26 (35)	4/10 (40)	22/68 (32)
Total	6/49 (12)	68/277 (25)	33/121 (27)	7/15 (47)	114/462 (24.7)

*Ann Arbor clinical staging classification (*8*).
†Rye histopathologic classification (*9*).
‡Number of primary relapses/total No. of cases treated, and (%).

tients with LP histology (12%); 68 among the 277 cases with NS disease (25%); 33 of 121 with MC (27%); and 7 of 15 (47%) with LD. There would thus appear to be surprisingly little difference in relapse rate with respect to clinical stage, but an appreciable increase in probability of relapse relative to histopathologic type, with LP being the least and LD being the most prone to relapse.

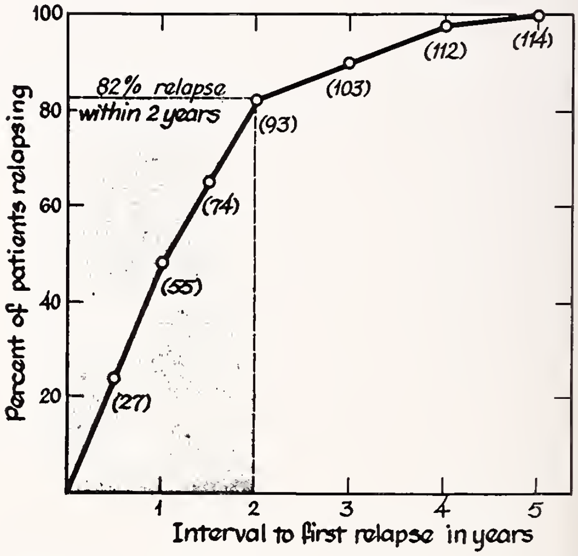
Sites of Relapse

There were a total of 160 sites of relapse among the 114 patients exhibiting primary relapses. Local relapses in initially involved, fully irradiated sites occurred, either alone or in conjunction with other sites of relapse, in a total of 19 instances, of which 2 had received IF, 6 EF, and 11 TN treatment. Regional relapses occurred in a total of 37 instances, either alone or in conjunction with other sites (14 IF, 8 EF, and 15 TN). Transdiaphragmatic relapses were observed in a total of 39 instances, alone or in conjunction with other sites (10 IF, 26 EF, and only 3 TN). Finally, extralymphatic relapses occurred in a total of 51 cases, alone or in conjunction with other sites; there were only 6 among the IF group, 11 in the EF group, and 34 in the TN group. However, no inferences concerning the apparently higher frequency in the TN group are justified, since a much higher proportion of the TN-treated cases were in Stage III and thus might have been expected to exhibit an increased probability of having their primary relapses in extralymphatic sites.

Analysis of the nodal sites of relapse with respect to their contiguity of relationship to the initially involved lymph-node chains, applying the criteria we described previously (*6, 14*), indicated that 43 of 54 nodal relapses (80%) were contiguous in distribution. Furthermore, an additional 10 relapses, some of which may have been marginal recurrences, occurred locally at a site of initial involvement which had been irradiated. Some of the extralymphatic sites of relapse may also have been consistent with the concept of contiguity. For example, the mediastinum was known to have been involved initially in 14 of the 17 patients with pulmonary parenchymal relapse; 8 of these also had ipsilateral or bilateral hilar lymph-node involvement. Such spread to the lung from mediastinal and/or hilar nodes is consistent with a contiguous pattern of spread. It is of interest that 4 of the patients who later developed pulmonary parenchymal relapses had received prophylactic whole-lung irradiation as a part of their initial treatment because of the presence of hilar adenopathy, receiving doses ranging from 1000 to 2200 rads in 1½–3 weeks. However, a larger number of patients will have to be studied after treatment with this technique before its usefulness in preventing pulmonary parenchymal relapse can be assessed.

Interval to First Relapse

The cumulative rate of appearance of relapses is indicated in text-figure 2 which shows that 48% of all of the relapses recorded in this study had occurred within the 1st year and 82% by the end of the 2d year. These figures are in excellent agreement with those previously published from this Institution (*1, 4*). By the end of 3 years, 90% of the relapses had occurred, and all patients in the series had suffered their primary relapse by the end of 5 years after the initial course of radiotherapy. The duration of follow-up for many of these cases was relatively



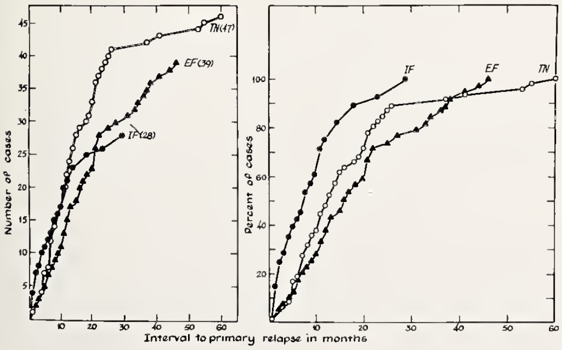
TEXT-FIGURE 2.—Cumulative percentage of patients showing interval to relapse.

short, and probably additional patients from the parent series will have relapses after 5 years. Nonetheless, the shape of the curve clearly indicates that a plateau was reached by 5 years, and it may be anticipated that the numbers of additional relapses after that time will be very small, in keeping with previous analyses (*1, 4, 15*).

The rate of accumulation of relapses among males and females did not differ significantly. Some tendency for longer relapse intervals to be concentrated in the 11- to 40-year age range was noted but was not significant. The interval to first relapse was not related to the duration of symptoms prior to the time of initial diagnosis.

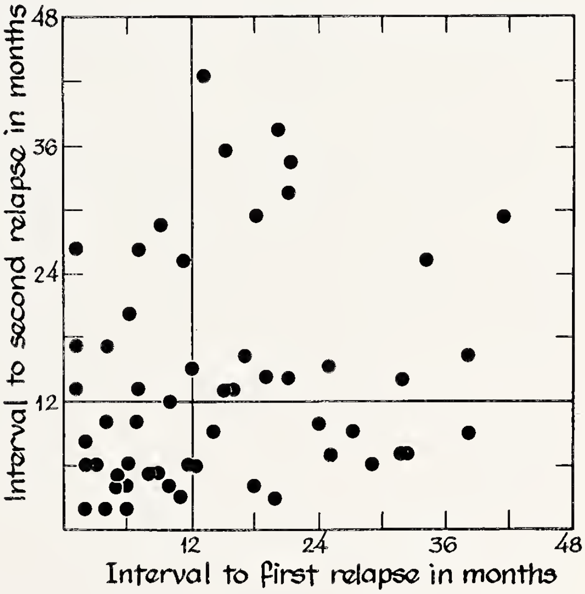
The influence of clinical stage and of constitutional symptoms on the interval to primary relapse is indicated in table 3. The mean interval to relapse was 19.3 months in Stage I-A cases, 16.9 months in Stage II-A, and 17.2 months in Stage III-A. In contrast, patients with constitutional symptoms (Stages I-B, II-B, and III-B) had mean intervals to relapse of 3.0, 12.0, and 14.4 months, respectively. The mean interval to relapse appeared to be somewhat shorter, in all stages, after IF treatment than after either EF or TN treatment. This was particularly apparent in the 7 Stage II-B patients treated with the IF technique, who had relapses after an average of only 4.7 months, with a range of 1–14 months. Cumulative relapse curves for each of these treatment techniques are presented in text-figure 3.

The mean interval to relapse was also influenced by the histologic type. It may be noted in table 4 that the 6 relapses in patients with LP histology occurred after a mean interval of 30.2 months (range 15–46 months); in NS, the mean interval decreased to 15.7 months (range 1–60 months), with further decreases to 13.9 months in the MC and to 9.3 months in the LD groups.



TEXT-FIGURE 3.—Cumulative relapse curves for the various treatment techniques.

Fifty-seven patients were available for analysis of the relationship between the time interval to first relapse and the interval to a second relapse. Although every combination of interval was noted, the majority had relapses for the second time at intervals consistent with a uniform tempo of disease (text-fig. 4). Of 29 patients, 19 (66%) whose first relapse interval had been <1 year relapsed for the second time within 1 year. Conversely, 17 of 28 patients (61%) whose first interval to relapse was >1 year had a second relapse which again occurred after an interval of >1 year.



TEXT-FIGURE 4.—Relationships between intervals to relapse.

INFLUENCE OF RELAPSE ON PROGNOSIS

Several groups of investigators previously reported the gravely adverse influence of a primary relapse after radiotherapy on ultimate prognosis (*1–4, 15*), and indicated that after-

TABLE 3.—*Influence of stage and constitutional symptoms on interval to primary relapse after radiotherapy and on post relapse prognosis*

Stage	Type of treatment	Number of patients	Interval to relapse (months)		Disease status*			
			Mean	Range	As̄	Ac̄	Dc̄	Di
I-A	IF	8	16.4	5–29	4	2	2	0
	EF	5	28.2	12–46	1	1	3	(1)
	TN	0	—	—	—	—	—	—
	Total	13	19.3	5–46	5 (38%)	3	5 (38%)	(1)
I-B	IF	0	—	—	—	—	—	—
	EF	1	(3.0)	—	0	0	1	0
	TN	0	—	—	—	—	—	—
	Total	1	(3.0)	—	0	0	1	0
II-A	IF	10	8.8	1–23	7	2	1	0
	EF	24	19.4	4–44	7	6	11	0
	TN	5	21.2	14–37	3	1	1	0
	Total	39	16.9	1–44	17 (44%)	9	13 (33%)	0
II-B	IF	2	4.7	1–14	0	2	4	1
	EF	7	16.0	2–34	0	1	6	0
	TN	11	14.2	5–53	4	1	6	(2)
	Total	25	12.0	1–53	4 (16%)	4	16 (64%)	1
III-A	IF	2	5.0	2– 8	0	0	2	(1)
	EF	1	33.0	—	0	0	1	(1)
	TN	11	18.0	4–60	2	2	7	(1)
	Total	14	17.2	2–60	2 (14%)	2	10 (71%)	(3)
III-B	IF	1	(2.0)	—	0	0	1	0
	EF	1	(2.0)	—	0	0	1	0
	TN	20	15.7	1–55	1	4	15	(1)
	Total	22	14.4	1–55	1 (4.5%)	4	17 (77%)	(1)
I, II, and III-A	Total	66	—	—	24	14	28	(4)
I, II, and III-B	Total	48	—	—	5	8	34	1
		114			29 (25.4%)	22	62 (54.3%)	1

*As̄ = alive without evidence of Hodgkin's disease; Ac̄ = alive with evidence of Hodgkin's disease; Dc̄ = dead with evidence of Hodgkin's disease; and Di = dead of intercurrent disease. Numbers in parentheses indicate cases also listed in the Dc̄ column because persistent Hodgkin's disease was either known to be present ante mortem or was discovered at autopsy in patients dying of other causes.

survival, computed from the time of relapse, is sharply reduced compared with that of other patients of initially similar stage who remain free of relapse for a corresponding time interval. This influence of relapse on prognosis is again apparent in the data of the present study. Analysis of survival, calculated from the time of first admission, indicated an overall survival of 53% at 5 years, with some tendency to flatten out at about 30% in the 7th and 8th years, though the numbers of cases remaining at risk at that time were small (table 5). The after-

TABLE 4.—*Influence of histologic type on interval to primary relapse after radiotherapy and on postrelapse prognosis*

Histologic type	Type of treatment	Number of patients	Interval to relapse (months)		Disease status*				% $D\bar{c}$
			Mean	Range	$A\bar{s}$	$A\bar{c}$	$D\bar{c}$	Di	
LP	IF	1	18	—	1	0	0	0	
	EF	4	37	21–46	1	1	2	0	
	TN	1	15	—	0	1	0	0	
	Total	6	30.2	15–46	2	2	2	0	33
NS	IF	18	8.1	1–29	9	3	5	1	
	EF	23	16.4	3–41	6	7	10	0	
	TN	28	19.6	4–60	9	6	13	(4)	
	Total	69	15.7	1–60	24	16	28	1	41
MC	IF	7	11.1	1–29	1	2	4	0	
	EF	10	19.7	2–38	1	0	9	(1)	
	TN	15	11.5	1–23	1	1	13	(1)	
	Total	32	13.9	1–38	3	3	26	0	81
LD	IF	2	6.5	2–11	0	1	1	0	
	EF	2	6.0	2–10	0	0	2	0	
	TN	3	13.3	7–25	0	0	3	0	
	Total	7	9.3	2–25	0	1	6	0	86

**Cf.* footnote to table 3.

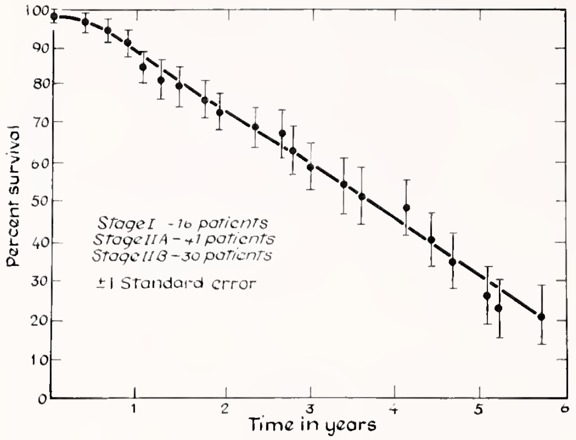
TABLE 5.—*Analysis of survival from date of admission in 114 patients with Hodgkin's disease, Stages I-III, developing primary relapse after radiotherapy*

Follow-up interval (yr)	Number of patients alive	Number of patients dead	Number of patients at risk	Survival* (%)
1	103	8	111	93
2	78	23	101	77
3	56	27	83	67
4	32	28	60	53
5	24	21	45	53
6	16	20	36	44
7	7	17	24	29
8	4	8	12	33
9	0	1	1	

*Uncorrected for intercurrent deaths.

survival of 87 of these patients whose initial extent of disease was limited to Stages I or II is presented in text-figure 5, which shows that 5-year survival from the time of the initial relapse was only 25% in this group of patients.

The interval to relapse, as noted previously (*16*), was also a factor in postrelapse prognosis (table 6), though the differences are not dramatic. Among the 59 patients whose primary relapse developed within 1–12 months, 61%



TEXT-FIGURE 5.—Survival of 87 patients with Stage I and II Hodgkin's disease after initial relapse.

were dead with disease and only 15 (25%) remained apparently disease-free at the time of reporting. Among the 35 patients whose interval to relapse increased to 13–24 months, 51% were dead with disease and 8 (23%) were apparently disease-free. Finally, among the 20 patients whose relapses occurred after ≧25 months, only 40% were dead with disease to date and 6 (30%) remained free of disease.

The influence of stage and constitutional symptoms on postrelapse prognosis is summarized in table 3 and that of histologic type on postrelapse prognosis in table 4. Although there were only 6 patients having relapses of LP histology, 2 (33%) of these remained apparently well, and 2 (33%) had died at the time of reporting. Of the 69 patients with NS histology, 28 (41%) are dead with disease and 24 (35%) were alive and apparently free of disease. Thus, these 2 histologic types offer some reasonable chance for salvage after a primary relapse. However, among the 32 patients with MC histology, 26 (81%) have already died, and only 3 (9%) have remained apparently free of disease at the time of reporting. As might have been expected, the 7 relapsing cases with LD histology have had the worst prognosis of all; 6 of the 7 have died with disease (86%), and the seventh case is alive with active, temporarily controlled disease.

The influence of constitutional symptoms on postrelapse prognosis appeared to be greater than that of stage. Thus, only 4 of 25 patients with Stage II-B disease who experienced a primary relapse (16%) are now apparently disease-free, and 64% have died, whereas 17 of 39 (44%) in Stage II-A are apparently disease-free, and only 13 (33%) have died with disease. The differences were less apparent in Stage III, where 71% of the "A" and 77% of the "B" cases have died with disease, leaving only 14%

TABLE 6.—*Influence of interval to primary relapse after radiotherapy on postrelapse prognosis*

Interval (months)	Type of treatment	Number of patients	Disease status*				
			$A\bar{s}$	$A\bar{c}$	$D\bar{c}$	Di	% $D\bar{c}$
1–12	IF	21	9	4	8	(1)	38
	EF	15	3	1	11	—	73
	TN	23	3	3	17	—	79
	Total	59	15	8	36	(1)	61
13–24	IF	5	1	1	2	1	40
	EF	13	3	3	7	(1)	54
	TN	17	4	4	9	(3)	53
	Total	35	8	8	18	1 (+4)	51
25+	IF	2	1	1	0	0	—
	EF	11	2	4	5	(1)	45
	TN	7	3	1	3	(2)	43
	Total	20	6	6	8	(3)	40

*Cf. footnote to table 3.

and 4.5%, respectively, free of disease in this more advanced group. Taking all asymptomatic cases together, there was a total of 66 cases of Stages I, II, and III-A, of which only 28 (42%) have died with disease, and 24 (26%) have remained apparently disease-free. Among the patients with constitutional symptoms (Stages I, II, and III-B), only 5 remained disease-free among a total of 48 (10%), and 34 have already died with disease (71%).

That the site of relapse also influences subsequent prognosis is evident in tables 7 and 8. It was readily apparent that about one-third to one-half of the local and regional relapses had apparently been salvaged, and currently remain disease-free, whereas the preponderance of transdiaphragmatic and, in particular, the extralymphatic relapses already proved fatal, and only 25% of the patients with the transdiaphragmatic relapses and 15% of those with extralymphatic relapses were apparently disease-free at this time. A more detailed analysis suggests that the pulmonary parenchyma may be a somewhat less unfavorable site of extralymphatic relapse than the liver, bone marrow, or subcutaneous tissues. Whereas all the patients with relapses occurring in bone marrow and virtually all the patients with relapses in the liver died, and all but one of the remaining patients with liver involvement had active disease, 4 of the patients with initial lung involvement remained apparently disease-free after multiple cycles of MOPP quadruple chemotherapy (a combination of mechlorethamine, vincristine, procarbazine, and prednisone) (table 9).

TABLE 7.—*Influence of site of relapse on postrelapse prognosis*

Major site of relapse	Disease status*				
	$A\bar{s}$	$A\bar{c}$	$D\bar{c}$	Di	Total
Local	2 (33)†	2	2 (33)	0	6
Regional	12 (42)	4	13 (45)	0	29
Transdiaphragmatic	8 (25)	7	16 (50)	1	32
Extralymphatic	7 (15)	8	32 (68)	0	47
Total No. and (%)	29 (24)	21	63 (56)	1	114

*Cf. footnote to table 3.

†Number of patients and (% of total in group).

Constitutional symptoms and histopathologic type both influenced the distribution of sites of relapse. Thus, extranodal sites of relapse occurred in 32 of 49 patients with constitutional symptoms (65%), but in only 18 of 65 asymptomatic patients (28%). Confirming the earlier observation of Keller et al. (*10*), the MC and LD types were more often associated with both noncontiguous and extralymphatic relapses. The latter occurred in 20 of 37 (54%) with MC histology and in 5 of 7 (71%) with LD histology, as contrasted with only 1 of 4 (25%) with LP and 24 of 66 (36%) with NS.

There were 10 cases of local relapse at an original site of disease (true recurrences), of which 7 were apparently attributable to a "marginal miss." By this, it is meant that disease was apparently present at the time of initial treatment immediately outside of the treated volume. Three of these patients had their initial relapses in the infraclavicular fossa; all had been treated before 1967, when this area was routinely shielded to protect the underlying lung. Since that time, the treatment technique has been modified to reduce the size of the anterior lung shields to deliver an adequate dose to the infraclavicular area, as described elsewhere (*11, 13*). In one patient with Stage III-A disease treated in 1962, the spleen area was not irradiated, though disease was documented in the upper para-aortic lymph nodes, and recurrence subsequently developed in the spleen. Two of the marginal misses were in the axillary region. The remaining 3 recurrences could be attributed to prolonged treatment times. The total dose of 4400 rads was ordinarily given, but the overall treatment time was prolonged for various reasons, to 7 weeks in 2 cases and to 13 weeks in 1 case (due to interruption by the necessity of thoracotomy.

Thus, there was no patient in this series in whom the only site of primary relapse was in an adequately treated area. There were, however, some examples of relapse in a previously treated area associated with simultaneous relapses at other, previously untreated sites. In these cases, there was at least the theoretical possibility of "reseeding" of the tumor back into the primary site from the site(s) of extension. Thus, the data substantiate the conclusion reached in earlier

TABLE 8.—*Multiplicity of site(s) of primary relapse after radiotherapy versus interval to relapse and postrelapse prognosis*

Type of treatment	Site(s) of relapse*	Number of patients	Interval to relapse (months)		Disease status†			
			Mean	Range	$A\bar{s}$	$A\bar{c}$	$D\bar{c}$	Di
IF	1	1	—	18	1	0	0	0
	2	11	6.0	1–14	7	1	3	0
	1 and 2	1	—	8	0	0	1	0
	3	8	15.1	4–29	2	4	2	0
	2 and 3	1	—	8	1	0	0	0
	4	4	7.0	1–23	0	1	3	0
	2 and 4	1	—	1	0	0	1	0
	3 and 4	1	—	14	0	0	0	1
	sites	=33						
	Total		9.4	1–29	11	6	10	1
	patients=28				(38%)		(36%)	
EF	1	2	7.0	6– 8	0	0	2	0
	2	6	20.8	9–38	2	1	3	(1)
	1 and 2	1	—	34	0	1	0	0
	3	19	20.4	3–46	4	2	13	(1)
	2 and 3	0	—	—	—	—	—	—
	4	3	15.0	7–22	1	1	1	0
	2 and 4	0	—	—	—	—	—	—
	3 and 4	4	20.2	2–37	1	0	3	0
	2 + 3 + 4	1	—	22	0	1	0	0
	1 + 3 + 4	2	22.5	4, 41	0	1	1	0
	1 and 4	1	—	2	0	0	1	0
	sites=	55						
	Total		18.8	2–46	8	7	24	(2)
	patients=39				(21%)		(62%)	
TN	1	3	19.3	15–22	1	2	0	0
	2	7	14.8	5–41	3	0	4	0
	1 and 2	1	—	10	0	0	1	0
	3	1	—	37	1	0	0	0
	1 and 3	1	—	9	0	1	0	0
	2 and 3	0	—	—	—	—	—	—
	4	20	14.7	1–53	3	1	16	0
	1 and 4	5	28.2	5–60	0	0	4	0
	2 and 4	6	19.3	7–55	1	2	3	0
	3 and 4	2	13.5	12, 15	1	1	0	0
	1 + 2 + 4	1	—	8	0	0	1	0
	sites=	72						
	Total		16.4	1–60	10	8	29	0
	patients=47				(21%)		(62%)	

*1 = local; 2 = regional; 3 = transdiaphragmatic; and 4 = extralymphatic.
†*Cf.* footnote to table 3.

studies (*5, 6*) that a dose of 4400 rads, given over a period of 4 weeks can consistently eradicate Hodgkin's disease within a treated field, with a true recurrence rate which is negligibly small, and no evidence is seen in the present study to support the concept that some cases of Hodgkin's disease are "radioresistant."

DISCUSSION

The data presented in this study provide further evidence to support the conclusion that the rate of development of relapses is related to histologic type. The relapse rate was 6 of 49 (12%) in LP cases; 68 of 277 (25%) in NS;

TABLE 9.—*Influence of site of primary relapse after radiotherapy on interval to relapse and on postrelapse prognosis*

Type of treatment	Site of relapse	Number of patients	Interval to relapse (months)		Disease status*				% Dc^{-}
			Mean	Range	As^{-}	Ac^{-}	Dc^{-}	Di	
IF	Local†	1	—	18	1	0	0	0	(—)
	Regional	13	7.4	1–23	7	2	4	0	(31)
	Transdiaphragmatic	10	14.3	4–29	3	4	2	1	(20)
	Extralymphatic	4	1.5	1–2	0	0	4	(1)	(100)
	Liver	1	—	1	0	0	1	0	
	Lung	1	—	2	0	0	1	0	
	Marrow	0	—	—	—	—	—	—	
	Subcutaneous	2	(1.5)	1, 2	0	0	2	0	
	Total	28	9.4	1–29	11	6	10	1	(36)
EF	Local	2	(7.0)	6, 8	0	1	1	0	(—)
	Regional	7	22.7	9–38	2	2	3	(1)	(43)
	Transdiaphragmatic	20	18.5	2–46	4	2	14	(1)	(70)
	Extralymphatic	10	17.4	2–37	2	3	5	0	(50)
	Liver	3	(21.3)	21–22	1	1	1	0	
	Lung	2	(11.5)	7, 16	0	1	1	0	
	Marrow	1	—	37	0	0	1	0	
	Subcutaneous	2	(12.0)	2, 22	1	0	1	0	
	Bone	1	—	22	0	1	0	0	
	Pleura	1	—	4	0	0	1	0	
	Total	39	18.8	2–46	8	8	23	0	(62)
TN	Local	3	19.3	15–22	1	2	0	0	(0)
	Regional	9	18.8	5–55	3	0	6	(2)	(67)
	Transdiaphragmatic	2	(23.0)	9, 37	1	1	0	0	(0)
	Extralymphatic	33	15.2	1–60	5	5	23	(2)	(70)
	Liver	10	8.2	1–15	0	2	8	0	
	Lung	9	21.6	11–53	4	1	4	(2)	
	Marrow	3	(21.3)	20–23	0	0	3	0	
	Bone	4	(8.0)	4–12	1	0	3	0	
	Subcutaneous	3	(23.5)	4–60	0	2	1	0	
	Pleura	2	(8.0)	7, 19	0	0	2	0	
	Central nervous system	2	(16.5)	8, 25	0	0	2	0	
	Total	47	16.4	1–60	10	8	29	0	(62)

*Cf. footnote to table 3.
†Cf. text for definitions of relapse sites.

33 of 121 (27%) in MC; and 7 of 15 (47%) in LD cases. The overall relapse rate was 114 of 462, or 24.7%. However, some of these cases had been followed for an inadequate time to expect a reasonably complete harvest of relapses, and it is likely that the overall relapse rate will continue to climb to at least a limited extent in the next 2–3 years.

Surprisingly, there was relatively little influence of clinical stage on overall relapse rate. Thus, there were 13 relapses among 64 Stage I-A cases at risk (20%); 39 of 164 Stage II-A and II$_E$-A (24%); 25 among 93 Stage II-B and II$_E$-B (27%); 14 among 71 Stage III-A, III$_E$-A, and III$_S$-A (20%); and 22 among 68 of III-B, III$_E$-B, and III$_S$-B (32%). Although the risk of developing a primary relapse was not significantly different among the various stages, both the site of initial relapse and the interval to relapse were influenced by stage and by the presence or absence of constitutional symptoms.

As previously reported, the occurrence of a primary relapse gravely influenced prognosis. and after-survival was sharply reduced compared with that of large groups of patients remaining relapse-free to the corresponding time interval. Extranodal relapses were associated with the most adverse prognosis, and transdiaphragmatic relapses within lymph-node chains were also more unfavorable than either regional or local relapses. The time interval to relapse was a factor in prognosis; patients with relapses occurring within the 1st year tended to develop a second relapse once again within 1 year and had a higher death rate than patients whose first relapse developed after 13–24 months or beyond 2 years. Nonetheless, even the most favorable group, those with relapses developing after ≧25 months in the local or regional sites, still had a very significant mortality, indicating that the occurrence of relapses after radiotherapy, even in sites near initially involved chains of lymph nodes, carries a grave prognosis, and that salvage rates leave much to be desired. Hopefully, the recent introduction of combination chemotherapy employing multiple drugs, supplemented in appropriate instances by low doses of radiotherapy, may significantly improve salvage in this unfavorable group of relapsing cases when further intensive radiotherapy is not possible.

The sites of relapse observed in the present study, when analyzed for contiguity of distribution by the criteria which we have previously described, once again provide strong support for the view that most new manifestations of disease in nodal sites tend to occur with a distribution pattern consistent with contiguity of spread via lymphatic channels. At least 80% of the nodal relapses documented in this study were consistent with such an interpretation, and an even higher percentage of contiguity is noted if extranodal relapses in the lung are scored in relation to the presence or absence of prior mediastinal and/or hilar adenopathy. However, there were also appreciable numbers of noncontiguous nodal relapses, in addition to relapses occurring in such extranodal sites as the liver and bone marrow. At least some of these are likely accounted for by adverse histology (MC and LD types) (*10*) and by the presence of vascular invasion, as previously reported by Rappaport and his associates (*17, 18*).

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Hodgkin's Disease: Survival and Relapse-Free Data[1]

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SUMMARY—The moderate-dose initial radiotherapy given at our hospital involves an undesirably high recurrence rate, but early and energetic management of relapse has shown that chances of retrieval are good. High-dose irradiation combined with long-term chemotherapy will certainly raise short-term survival rates but will entail overtreatment of many patients. Optimal first treatment requirements should ideally be assessed for each individual or at least for small homogeneous groups of patients. The risk taken in giving something less than the maximum initial treatment (accepting a particular level of recurrence rate) can be estimated from the retrieval rate obtained when patients are treated for recurrence. The risk taken in raising radiation doses or in giving long courses of chemotherapy can be estimated from the ill effects of such treatment on survivors. Increasing serious damage done by maximal treatment must be weighed against decreasing small improvements in survival rate.—Nat lCancer Inst Monogr 36: 509–512, 1973.

DATA on survival and relapse have been recorded for all patients with Hodgkin's disease attending the Royal Marsden Hospital. The figures in the tables include all previously untreated patients first seen in the periods stated, but not all these patients attended our Lymphoma Unit: The investigation and treatment of some of them have not been entirely in our hands. The results presented therefore follow less uniform management than has been the case at Stanford University School of Medicine. The Lymphoma Unit started lymphography in 1962 and made it a routine pretreatment investigation for Hodgkin's disease in 1963. We have used extended-field irradiation for all our patients since 1963, so we were 1 or 2 years behind Stanford in applying present treatment regimens; the results in our earlier group of patients with the longer follow-up have been the least satisfactory (text-fig. 1, table 1).

It seemed, therefore, that to follow the Stanford group with our slightly worse relapse-free data—even though showing the same marked rise in survival rate—would not add much to our discussion.

Our treatment has, in the past, been less radical than that given at Stanford, which accounts for our higher recurrence rate; on the other hand, we have treated relapse energetically and have had rather longer experience than Stanford of intensive spaced, quadruple chemotherapy, as already reported by McElwain (*1*). I have therefore arranged our data so as to give a view of the chance of retrieval, once recurrence has appeared.

We could all be fairly certain of raising short-term survival rates in Hodgkin's disease if we were to give total nodal irradiation and 6 courses of quadruple chemotherapy to every patient, but if we were to do so, prolonged and damaging treatment would be given unnecessarily to the majority of patients. We have yet to learn what length of life and what mode of death await those patients cured of Hodgkin's disease who have had a splenectomy, extensive

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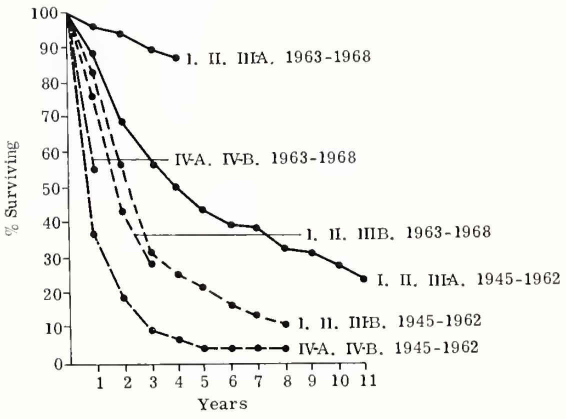
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

Table 1.—*Hodgkin's disease, all stages, male and female: Results of treatment by histologic type at the Royal Marsden Hospital, 1963–70*

Survival after treatment (yr)	Lymphocytic predominance	Nodular sclerosis	Mixed cellularity	Lymphocytic depletion	Number of patients at risk
	Patients (%)	Patients (%)	Patients (%)	Patients (%)	
1	21/23 (91)	99/103 (96)	43/53 (81)	12/19 (63)	198
2	16/18 (89)	73/79 (92)	26/38 (68)	6/16 (38)	151
3	9/11 (82)	50/59 (85)	19/32 (59)	3/13 (23)	115
4	5/7 —	33/39 (80)	9/22 (41)	1/10 (10)	78
5	3/3 —	21/27 (78)	6/15 (40)	0/7 —	52



Text-figure 1.—Hodgkin's disease: Survival after treatment by stage at the Royal Marsden Hospital. Results for 1963–68 compared with those for 1945–62.

bone-marrow irradiation, some radiotherapy damage to lungs and heart, and the added rigors of prolonged chemotherapy. If we are to reduce risks without prejudicing chances of survival, we must eventually make a firm assessment of the treatment requirements of each patient, or at least of the optimal treatment for small groups of patients subdivided by histology, sex, age, and extent of disease. To judge the risks we are entitled to take in giving less than the maximum treatment, we must estimate the retrieval rate obtainable in those patients who develop recurrence after the less drastic treatments have been given. If patients in a defined group (women of 45 years or under in Stages I, II, and III, for example, table 2) have a 75% recurrence-free rate with a safe but restricted treatment regimen, and if 60% of those with recurrence (15% of the total) are retrievable by subsequent treatment, then 90% will be cured. If maximum treatment would have secured a 95% cure rate from the start but introduced greater hazards for all patients, then 75% would have been overtreated for long periods and subjected to unnecessary risks and perhaps to a mortality from the treatment for the sake of the 5% who might not otherwise have been saved. Each group of patients has its special treatment needs and runs special risks from overtreatment. A study of retrieval rates may show what proportion of patients really benefit from treatment and may help us to put more discrimination into the planning.

Tables 3 and 4 present the worst possible view of our recurrence rate. All patients requiring treatment for the disease, however trivial, after their first planned treatment have been counted as recurrent cases. Most of these treatments were given below the diaphragm to patients who started with normal lymphograms and were given mantle irradiation but subsequently showed involvement of para-aortic nodes. Some patients, however, had recurrence at the edge of treatment fields (in the preauricular region or elsewhere), and some had widespread dissemination. These patients have all been observed for a minimum of 2 years since treatment, so that most of the likely recurrences have probably been included.

Our data support the view that good retrieval is possible in recurrent cases if they are found early on regular follow-up visits, and that the extent of the first planned treatment should take

TABLE 2.—*Hodgkin's disease, Stages I–III: Survival of women, aged 45 years and under, treated at the Royal Marsden Hospital, 1963–70*

Ann Arbor clinical stage	Number of patients treated	Number of patients surviving for the following No. of years:							Number of patients dead
		1	2	3	4	5	6	7	
I-A	8	1	3	1	2	1	—	—	—
II-A (2)	21	2	3	4	4	6	1	1	—
II-A (3 & 4)	11	1	3	—	3	1	1	1	1
II-B	8	1	2	—	3	1	—	1	—
III-A	6	—	4	—	—	1	1	—	—
III-B	5	—	3	—	1	—	—	—	1
Total	59	5	18	5	13	10	3	3	2

TABLE 3.—*Hodgkin's disease, Stages I and II: Survival and recurrence by absence or presence of systemic symptoms and by sex. Patients treated at the Royal Marsden Hospital, 1963–69, and observed for 2–8 years*

Ann Arbor clinical stage systemic symptoms/ sex	Number of patients	Number of patients with recurrences (%)	Number of patients dead	Number of patients surviving (%)
Stages I and II, A and B, male and female	108	49 (45)	15	93 (86)
Stages I and II, A and B, male	59	35 (59)	14	45 (76)
Stages I and II, A and B, female	49	14 (28)	1	48 (98)
Stages I and II, male and female, A	86	38 (44)	11	75 (87)
Stages I and II, male and female, B	22	11 (50)	4	18 (82)

this into consideration. A few more years of observation of these patients, and the results of trials of extended mantle ("anchor") treatment now in progress should give us a more secure footing on which to base our treatment plans and obtain the greatest benefit for the least risk.

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Table 4.—*Hodgkin's disease, Stages I–III, systemic symptoms absent and present: Survival and recurrence by stage and sex. Patients treated at the Royal Marsden Hospital, 1963–69, and observed for 2–8 years*

Ann Arbor clinical stage systemic symptoms/ sex	Number of patients	Number of patients with recurrences (%)	Number of patients dead	Number of patients surviving (%)
Stages I and II (2), A and B, male and female	73	30 (41)	7	66 (90)
Stages I and II (3, 4, and 5), A and B, male and female	35	19 (54)	8	27 (77)
Stage III, A and B, male and female	38	23 (61)	II	27* (71)
Stage III, A and B, male	29	18 (62)	9	20 (69)
Stage III, A and B, female	9	5 (56)	2	7* (78)

*One patient lost sight of.

Survival After Recurrence: Prognostic Factors and Spread Patterns in Clinical Stages I and II of Hodgkin's Disease[1]

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SUMMARY—Two series, about 450 cases, of patients with clinical Stage I and II Hodgkin's disease were analyzed for prognostic factors and patterns of presentation. Many patients with the histologic type of nodular sclerosis survived for a long time, even after disease recurrence. Long-term chemotherapy after radiotherapy tended to decrease the incidence of recurrence in nonirradiated lymph-node areas. Multiple regression analysis demonstrated the predominant influence of histologic type and sex on survival; the erythrocyte sedimentation rate also had some prognostic value. An empirical prognostic index was described and tested. Patients with mediastinal involvement survived significantly longer. Mediastinal involvement was correlated with histologic type, sex, and age. Incidence was higher in women between 15 and 50 years. The prognosis of patients with mediastinal involvement was good (even for men and those with mixed-cellularity histologic type Hodgkin's disease), and the frequency of transdiaphragmatic lymph-node recurrence was low. Patients without mediastinal involvement, in particular those with mixed-cellularity histologic type, had higher recurrence rates. Natl Cancer Inst Monogr 36: 513–530, 1973.

DURING RECENT YEARS, there has been remarkable progress in treatment of Hodgkin's disease patients with clinical Stages I and II. Five-year survival rates of 80%–90% are now common (*1, 2*). This progress is mainly due to the extension of the target volume treated by radiotherapy, to the use of chemotherapy after radiotherapy, and, to an extent difficult to evaluate, to splenectomy.

However, good results were formerly obtained with localized radiotherapy, especially in favorable groups (*3, 4*). Thus many Stage I and II patients, particularly the young, are being overtreated. Furthermore, radical treatments are not without risk. The somatic hazards of total-nodal radiotherapy are obvious although difficult to estimate. Radiotherapy places a genetic burden on the young patients especially. Computations carried out at the Institut Gustave-Roussy indicate that about 250 rads of scattered radiation is received by the testes during an inverted Y-field treatment by telecobalt. If not transplanted, the ovaries receive the full dose. If moved behind the uterus in the midline, they receive about 400–800 rads; if transplanted in the iliac region, about 400. Even during the irradiation of the para-aortic nodes, the doses are about 200 rads for the testes and 300 rads for the ovaries.

The somatic and genetic effects of chemotherapy are not as well known but are probably at least as important as those of total-nodal irradiation. For instance, azoospermia and a high incidence of amenorrhea occur after intermittent quadruple chemotherapy (*5*).

The effect of splenectomy on immunologic defenses and lifespan is difficult to evaluate in humans without specially designed and controlled clinical trials.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

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On the other hand, patients treated with localized radiotherapy have frequent relapses affecting previously unirradiated and uninvolved areas. Some data (*6*) suggest that the chance of long-term survival diminishes after one recurrence; restricted field therapy may therefore lower a patient's chances for cure.

To find a balance for the risks of overtreatment and the hazards of undertreatment, quantitative studies would be valuable. Follow-up data on patients treated with localized therapy could illustrate patterns of recurrence and spread. Comparisons of the courses of patients given localized and more radical treatments would indicate some potential risks of undertreatment, especially if the treatment of relapses were standardized.

An unequivocal answer could be provided by a controlled clinical trial, similar to the study of Paterson and Russell (7) of postoperative irradiation in breast cancer, in which half the patients were given prophylactic irradiation of the chest wall but the other half were not, except after a recurrence. Paterson and Russell found no difference in survival between the 2 groups. Such a clinical trial would be ethically acceptable only for those types of the disease in which the survival rate appears to be similar after either prophylactic treatment of noninvolved areas, or initially localized radiotherapy followed, if recurrence does occur, by a more aggressive treatment. In other words, the trial should be restricted to those types of patients in whom long-term survival can still be expected after a first recurrence.

To prepare the grounds for such trials and to learn more about the course of the disease in patients given modern treatment, we studied the factors which influence survival after the first recurrence, the duration of the first remission, and the rate of survival after the first treatment. Since most of the prognostic factors are interrelated, multiple regression techniques were used to assess the independent prognostic value of each factor considered.

The prognostic factors seemed to vary with the type of treatment, and our analysis was based on 2 series of patients: *a*) Patients with clinical Stages I and II of Hodgkin's disease treated at the Institut Gustave-Roussy between 1922 and 1963. This series was discussed in (*8*). Basic clinical and biologic data were available for 160 patients with clinical Stages I and II, and the prognostic study was carried out on these patients. *b*) Patients included by Cooperating Centers in the work on Hodgkin's disease by the European Organization for Research and Treatment of Cancer (E.O.R.T.C.). Results were discussed in (*9*) as well as at this Symposium.

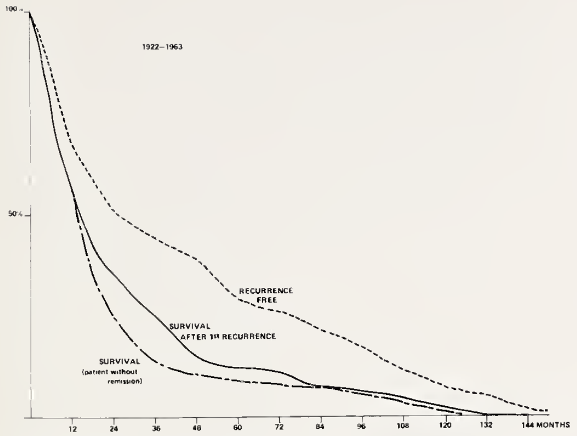
SURVIVAL AFTER FIRST RECURRENCE

Hodgkin's Disease Treated Between 1922 and 1963 in Villejuif

In 82 of 160 Stage I and II patients considered, no complete remission was reached after the first treatment. Survival was 30.4±6.4 months (mean ± 95% confidence interval). In 76 patients, there was complete remission after the first treatment, with a first remission that lasted 30.5±6.6 months, and a mean duration of 46.7±11.4 months between first recurrence and death.

Text-figure 1 shows the percentage of survival and of recurrence-free patients in the 2 groups. The shapes of the survival curves for the patients without remission after treatment and for patients who were in complete remission after treatment are strikingly similar. For the patients who attained complete remission after treatment, we found no correlation between duration of first remission and survival after first recurrence. The data show that, for patients treated when treatments were generally palliative, the survival after recurrence is not related to either the duration of the first remission or to the initial course of the disease and previous treatments.

Of the 296 patients studied by the E.O.R.T.C., 96 had relapses. For those, the mean duration of the recurrence-free period was 24.8±3.1 months for patients given radiotherapy alone and 29.3±3.3 months for those treated by radiotherapy followed by long-term chemotherapy; the difference between these 2 durations is significant at 0.05. Forty-nine patients died. For

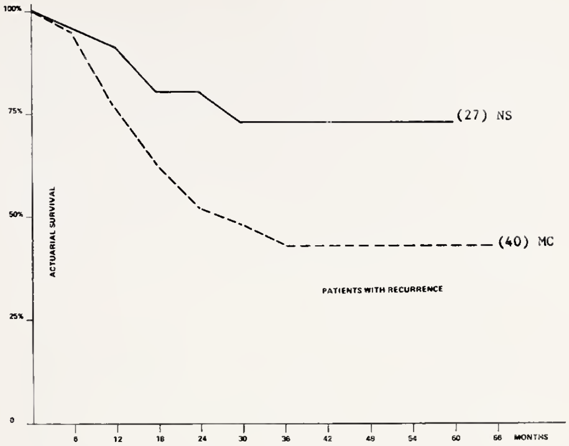


Text-figure 1.—Percentage of survival (actuarial) in 2 groups of clinical Stages I and II of Hodgkin's disease treated between 1922 and 1963 (*12*). The first group (*upper* and *middle curves*) concerns 76 patients in whom complete remissions were obtained after the first treatment. The *upper curve* indicates the percentage of recurrence-free patients and the *middle curve* the percentage of patients surviving after the first recurrence. The second group concerns 82 patients in whom a complete remission was not obtained. The *lower curve* indicates their survival.

these, the mean interval between treatment and recurrence was 12.8±3.92 months.

Follow-up studies of patients after recurrence have been relatively recent, and it may seem premature to study their survival. Nevertheless it seems that many long-term survivals, equivalent to cures, can be expected. This is especially true of patients with nodular sclerosis (NS) histologic types in whom we may hope for about 70% long-term survivals after recurrence (text-fig. 2). This probability seems smaller for patients with mixed-cellularity (MC) type. The probability of survival after recurrence is less for patients with mixed cellularity. This difference between the two histologic types is already apparent when one compares both the percentage of patients who are recurrence-free after first treatment and the percentage of survivors.

The survival curve after recurrence also seems to be influenced by presence or absence of systemic symptoms before the first treatment and by the site of the recurrence (better for recurrence in an irradiated area than for transdiaphragmatic nodal recurrence and worse for extranodal recurrence). On the other hand, the initial treatment (radiotherapy alone or radiotherapy followed by vinblastine) does not seem to influence survival after recurrence, although the first remission lasts slightly (but significantly) longer in the group treated by combination therapy.



Text-figure 2.—Survival (calculated by the actuarial method) after first recurrence in the E.O.R.T.C. series (clinical Stages I and II) for 27 patients with NS and 40 patients with MC.

Prognostic Factors

A. A previous paper (*8*) discussed prognostic factors in a retrospective series of 454 patients with Hodgkin's disease initially treated in 1922–63. Most patients were treated by localized conventional radiotherapy, and radiation doses were moderate. Therefore, it seemed interesting to compare the prognostic factors identified in this series with those influencing the course of the disease for the E.O.R.T.C. patients given higher doses and more extensive radiotherapy, followed sometimes by long-term chemotherapy.

In both series, the histologic type, age, and sex are significant to the length of remission and to survival rates. In the retrospective series, the results were influenced by the stage—Stages I($_1$), I($_2$), and II of the Rye-Paris classification. No significant influence of the stage on the duration of remission or on survival was noted in the E.O.R.T.C. series. Moreover, in this series, a long follow-up shows a slightly greater tendency toward better survival for Stage II than for Stage I patients. Hence the influence of stage on prognosis recedes when the areas contiguous to the involved ones receive a prophylactic irradiation. Since transdiaphragmatic lymph nodes were not prophylactically irradiated in the E.O.R.T.C. series, the tendency toward a better prognosis for Stage II patients suggests that transdiaphragmatic, clinically unsuspected involvement may be more frequent in Stage I than in Stage II.

Systemic symptoms had a high prognostic significance in the retrospective series. They are not important to the duration of the first remission in the E.O.R.T.C. series but do influence survival after recurrence. The analysis of the prognostic value of the erythrocyte sedimentation rate (ESR) and of systemic symptoms illustrates the relationship between type of treatment and prognostic factors. The patients can be divided into 4 subgroups according to the presence or absence of systemic symptoms and to the normal or accelerated ESR value. In the retrospective series, the group without systemic symptoms and with ESR <30 mm/hour has a significantly higher survival rate than do the 3 other groups. In the E.O.R.T.C. series, preliminary data suggest that survival is lower in the group with systemic symptoms and an accelerated ESR (table 1). Furthermore, of the E.O.R.T.C. patients treated by radiotherapy alone, those with accelerated ESR have more recurrences. Patients treated by radiotherapy and chemotherapy have a lower overall incidence of relapses, not significantly correlated with the ESR value (table 2). Thus an accelerated ESR may be correlated with a higher probability of an early spread to those noncontiguous areas not included in the treatment fields. The difference between the 2 therapeutic groups may be explained by chemotherapy's ability to delay relapses in the nonirradiated transdiaphragmatic zones. In fact, multiple regression analysis shows that initial ESR has an independent and significant prognostic value for predicting a recurrence during the first 2 years for patients treated by radiotherapy alone and, at the 4th year, for patients treated with combined radiotherapy and long-term chemotherapy.

TABLE 1.—*E.O.R.T.C. series*

ESR	Number of patients	% recurrence-free patients at 4 years	% survival at 4 years
A. <30 mm/hr	175	55	80
A. >30 mm/hr	67	50	80
B. <30 mm/hr	18	60	80
B. >30 mm/hr	16	30	30

B. Table 3 gives the correlations between the various factors studied in the E.O.R.T.C. analysis. In the retrospective study (*8*), most of the factors are interrelated (text-fig. 3), which makes it difficult to assess the independent prognostic significance of each.

Multiple regression analysis, which considers these correlations, is a useful tool. Results of the computations are shown in tables 4A and B.

For patients in the retrospective series who never achieved remission, the histologic type is the most significant factor during the follow-up. For patients in this series in whom the first treatment produced a complete remission, histologic type is the preeminent factor during the first 2 years. Later, age has a slight significance,

TABLE 2.—*E.O.R.T.C. series—patients followed for >3 years*

Therapy	ESR	Patients without recurrence	Patients with recurrence	Number of patients	
Radiation	<30 mm/hr	66%	34%	101	S* <0.02
	>30 mm/hr	45%	55%	42	
	Number of patients....	86	57	143	
Radiation + vinblastine	<30 mm/hr	75%	25%	91	NS†
	>30 mm/hr	68%	32%	41	
	Number of patients....	96	36	132	

*Significant.
†Not significant.

TABLE 3.—*Correlation between prognostic factors—E.O.R.T.C. series*

	Clinical staging	Histology	Systemic symptoms	Age	Sex	ESR	Med*
Clinical staging	//////////	NS†	$P<0.01$	NS	NS	<0.005	<0.001
Histology	//////////	////////////	NS	<0.05	0.02	NS	<0.001
Systemic symptoms	//////////	////////////	/////////	NS	NS	<0.02	NS
Age	//////////	////////////	/////////	//////	NS	NS	NS
Sex	//////////	////////////	/////////	//////	//////	NS	<0.001
ESR	//////////	////////////	/////////	//////	//////	///////	<0.001

*Mediastinum involvement.
†Not significant.

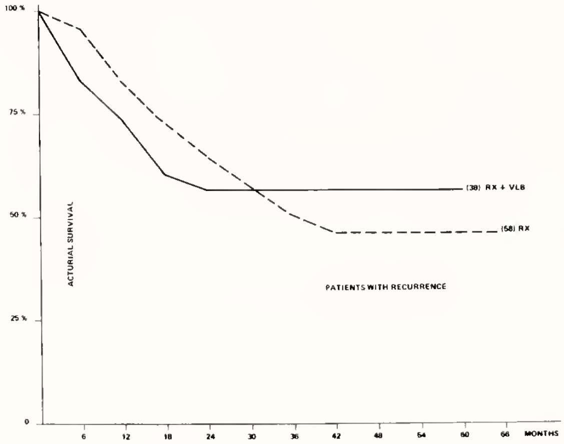
and sex becomes the most important single factor.

In the E.O.R.T.C. series, histologic type remains the most significant factor during the course of the disease. An independent prognostic value is shown by age during the first 2 years, and later by sex (tables 4A, 4B).

In summary, multiple regression analysis underlines the fact that, for clinical Stages I and II, histologic type is the most important single prognostic factor. Sex has some independent prognostic value.

C. For many diseases one could use formulae incorporating the various prognostic factors (*9, 10*) with different weighting factors. Values were assigned to the factors considered and were chosen empirically to fit the survival data in the 1922–63 series (table 5).

Text-figures 4A and 4C give the percentage of recurrence-free patients for patients staged using the set of values indicated in table 5 which we refer to as the "code." In the 1922–63 series, the duration of the first remission is not predictable from the various code values. On the other hand, the code is of prognostic value for the duration of remission in the E.O.R.T.C. series.



Text-figure 3.—Survival after first recurrence in the E.O.R.T.C. series (clinical Stages I and II) for 38 patients treated by radiotherapy (RX) with long-term chemotherapy and 58 patients treated by RX alone. VLB = vinblastine.

In retrospect, the formula would have been of great prognostic value for the 1922–63 series survival data (text-figs. 4B, D) when a complete remission had been initially obtained. For the E.O.R.T.C. series, it would have had similar prognostic value. For the group with the best prognosis (code O), the survivals were similar in the 2 series, though the duration of the first remission was shorter in the retrospective group. On the other hand, for other code values, remission and survival are much better in the E.O.R.T.C. series, probably as a result of the more aggressive treatment.

With multiple regression analysis, theoretical values for each of the parameters of the prognostic index can be calculated. Although the predictions based on these calculated values fit the survival data on which they were estimated much better than the predictions based on the empirically chosen values, when these calculated values are used for other groups of patients, the resulting predictions are less satisfactory than those based on the chosen values. This problem will be discussed in another paper.

D. Comparison between the 2 types of treatments used on E.O.R.T.C. patients can help to clarify the meaning of certain prognostic factors and to assess the various patterns of the spread of the disease.

The E.O.R.T.C. report pointed out that

Table 4A.—*Retrospective series—Stages I and II (patients in complete remission after treatment; multiple regression analysis of prognostic factors)—when significant; the level of significance is indicated*

	Years	Stage	Histologic type	Systemic symptoms	Age	Sex
Survival	2		$P<0.02$			$P<0.10$
	3					
	4	$P<0.10$				$P<0.05$
Recurrence-free	2					
	3					$P<0.05$
	4					$P<0.02$

TABLE 4B.—*Study of prognostic factors by multiple regression (E.O.R.T.C. series)*

	Years	Clinical stage	Histologic type	Age	Sex	Systemic symptoms	ESR	Involvement of mediastinum	Treatment*
Survival	2	1.72† ($P<0.10$)	2.52 ($P<0.05$)	1.88 ($P<0.10$)	0.95	1.02	0.73	0.97	1.42
	3	0.50	2.68 ($P<0.01$)	1.48	1.33	0.46	0.35	1.41	0.22
	4	0.18	3.34 ($P<0.001$)	1.73 ($P<0.10$)	2.13 ($P<0.05$)	0.57	0.37	0.82	1.09
Recurrence-free	2	0.37	2.75 ($P<0.01$)	2.62 ($P<0.01$)	1.87 ($P<0.10$)	0.14	1.46	0.65	2.39 ($P<0.05$)
	3	0.21	3.24 ($P<0.01$)	1.24	2.89 ($P<0.01$)	0.22	0.006	0.03	0.67
	4	1.82 ($P<0.10$)	2.45 ($P<0.05$)	1.32	2.41 ($P<0.05$)	0.10	0.85	0.03	0.18

*The 2 therapeutic subgroups (radiotherapy alone; combination).

†The number refers to the statistical significance calculated by multiple regression analysis. When significant, the level of significance is indicated below.

Table 5.—*Numbers of patients: In retrospective series, 84 for patients in complete remission after first treatment, 97 for patients without complete remission. In the E.O.R.T.C. series, 180 patients*

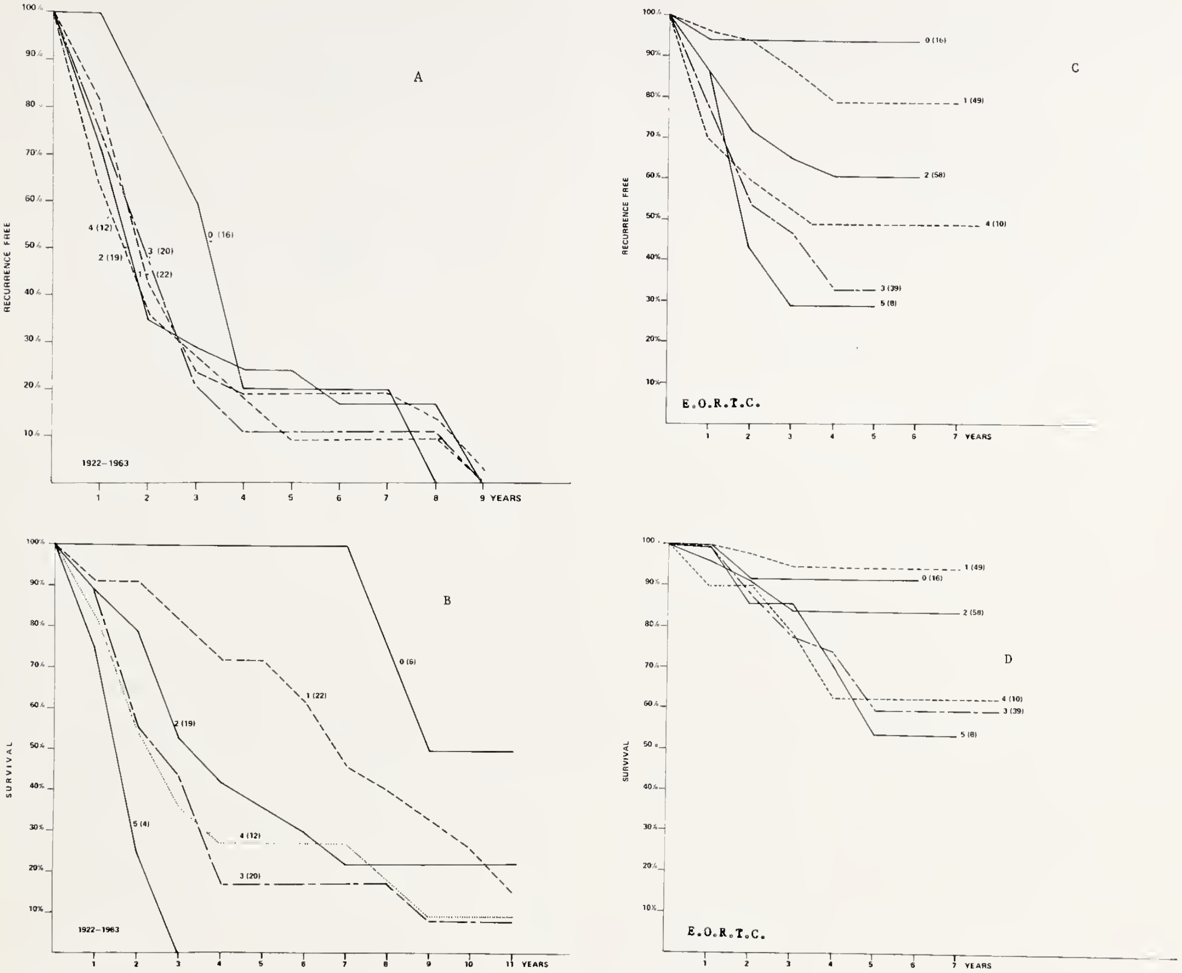
		Retrospective series			Code	E.O.R.T.C. series	
		Patients in complete remission after first treatment		Patients without complete remission after first treatment: % survival at 3 years	Chosen value for prognostic index	% recurrence-free at 3 years	% survival at 3 years
		% recurrence-free at 3 years	% survival at 3 years				
Stage	I($_1$)	37	77	51	0	61	80
	I($_2$) + II	15	51	23	1	59	88
	III + IV	29	33	15	2	NS	NS
		+	+	+			
Histologic type	NS	18	71	40	0	74	93
	LP*	22	55	42		88	94
	MC	26	45	13	1	44	74
	LD†	20	17	9	2	31	83
		NS‡	+§	+		+++‖	NS
Systemic symptoms	Absence	28	62	30	0	60	85
	Presence	18	43	20	1	63	87
		NS	NS	NS		NS	NS
Age (yr)	<40	27	61	23	0	65	86
	⩾40	23	46	27	1	38	80
		NS	NS	NS		+	NS
Sex	Female	32	71	36	0	77	92
	Male	20	43	19	1	51	81
		NS	+	NS		++¶	NS

*Lymphocyte predominance.
†Lymphocyte depletion.
‡Not significant.
§+ = $P<0.05$.
‖+++ = $P<0.001$.
¶++ = $P<0.01$.

addition of long-term chemotherapy to extended radiotherapy results in a longer mean first remission and a higher survival rate (*11*). The number of recurrences in an irradiated area is small in either treatment group. However, the incidence of recurrence in an unirradiated area is significantly lower in the group receiving chemotherapy (31 recurrences among 155 patients treated by radiotherapy; 14 recurrences among 141 patients treated by radiotherapy and chemotherapy). Surprisingly the incidence of extranodal and systemic relapses does not differ in the 2 groups (table 6).

The effect of chemotherapy shows mainly in the MC group [table 7, (*11*)]. It could be asked whether the MC is especially sensitive to vinblastine or if its natural history is different from that of the other histologic types.

Of 130 patients with LP- or NS-type Hodgkin's disease, 16 had relapses in a nonirradiated area. Of 107 patients with MC or LD types, 22 had relapses in a nonirradiated area. This



TEXT-FIGURE 4.—Evaluation of the prognostic index (table 5). A.—Recurrence-free percentage (calculated by the actuarial method) in the retrospective group for the 76 patients who reached complete remission (*8*). Numbers correspond to the value of the prognostic index (table 5). *Numbers in parentheses* indicate patients in each subgroup. B.—Survival (actuarial) in the retrospective group for the 76 patients who reached complete remission. C.—Recurrence-free percentage in the E.O.R.T.C. series (clinical Stages I and II). D.—Survival in the E.O.R.T.C. series.

type of recurrence is therefore nearly twice as frequent in the latter histologic types (table 7). On the other hand, in both types, the incidence of this pattern of recurrence in patients treated with radiotherapy alone is about 2 times greater than that in patients also given chemotherapy (10 vs. 6 for LP and NS; 15 vs. 7 for MC and LD).

It seems therefore that the various histologic types are about equally sensitive to vinblastine and that the effectiveness of chemotherapy is related to a higher propensity for transdiaphragmatic lymph-node involvement in the MC and LD histologic types.

The effect of long-term chemotherapy with vinblastine can also be studied in relation to the other prognostic factors. In general, vinblastine seems most effective in the worst prognostic group. It is more useful in patients >30 years old than in younger patients and in men more than in women. The usefulness of chemotherapy is influenced by sex even within the same histologic type. For instance, in the MC type, radiation and vinblastine improve results in men but not in women. On the other hand, clinical stage and presence or absence of systemic symptoms do not seem to significantly influence the effectiveness of vinblastine. But the most striking

TABLE 6.—*Percent recurrence compared between the 2 groups according to site of recurrence (E.O.R.T.C. series)*

	Radiotherapy	Radiotherapy + vinblastine	Total
Patients at risk	155	141	296
Recurrence in irradiated area	6 (3.9%)	5 (3.5%)	11
Recurrence in nonirradiated area	31 (20%)	14 (9.9%)	45
	$P<0.02$		
Recurrence—visceral, systemic, or biologic	21 (13.5%)	19 (13.5%)	40

TABLE 7.—*Incidence of recurrence: E.O.R.T.C. series*

		LP + NS	MC + LD	Histologic type unknown	Total
Recurrence in irradiated area	RX*	2 (6%)	3 (6.5%)	1	11
	RX + VLB†	1 (3%)	1 (2%)	3	
Recurrence in non-irradiated area	RX	10 (30%)	15 (32.5%)	6	45
	RX + VLB	6 (18%)	7 (15%)	1	
Recurrence—visceral, systemic, or biologic	RX	8 (25%)	10 (22%)	3	40
	RX + VLB	6 (18%)	10 (22%)	3	
Total No. recurrences		33 (100%)	46 (100%)	16	95
Number patients at risk		130	107	59	296

*Radiation therapy.
†Vinblastine.

observation is that chemotherapy is much more useful in patients without, rather than with, mediastinal involvement. This raises the problem of the significance of mediastinal involvement and of the natural history of Hodgkin's disease.

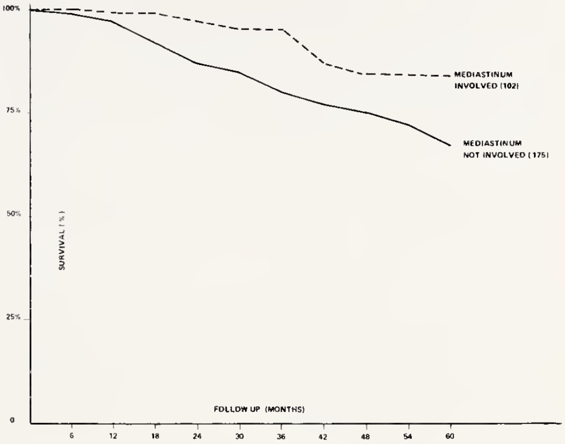
MEDIASTINAL INVOLVEMENT AND PATTERN OF PRESENTATION

Influence on Prognosis of Mediastinal Involvement

In 1966, Papillon et al. (*12*) noted that a high proportion of patients with initial mediastinal involvement were among long-term survivors. In 1971, Peters (*2*) found that among patients treated between 1931 and 1960 [clinical stages $I_{(2)}$ and II of the Rye classification], the mean recurrence-free interval was 5 years in patients with mediastinal involvement as against 1 year for patients without mediastinal involvement; the median survival was 17 years and 7.5 years, respectively. Moreover, Peters observed that for Stage II-A, in presentations with mediastinal involvement, the survival rates are comparable regardless of the number of regions involved, whereas in all other Stage II-A presentations, the survival rates are much lower if >2 contiguous regions are involved. Conversely, for Stage III, in the prelymphographic era, she found that the survival rates were distinctly better in the group without mediastinal involvement, and she suggested that Stage III cases with mediastinal involvement are in late Stage III. She concluded that the mediastinum is a key disease site. When she wrote this paper, it was known that mediastinal involvement was highly correlated with NS type, but she could not assess the independent prognostic value of the 2 parameters: histologic type and presentation.

In the retrospective series (*8*) patients with clinical Stage $I_{(1)}$ had better survival rates when the mediastinum was involved than when it was not. No difference was noted in other stages, contrary to Peters' findings, perhaps because the radiation therapy was too localized.

In the E.O.R.T.C. series, the survival rate was significantly higher in patients with mediastinal involvement than in other patients (text-fig. 5). However, since mediastinal involvement is relatively more frequent among women and among patients with NS type, these associations must be considered for prognostic significance.



TEXT-FIGURE 5.—Survival (actuarial) for patients with or without mediastinal involvement. E.O.R.T.C. series (clinical Stages I and II).

Correlation Between Histologic Type, Age, Sex, and Mediastinal Involvement

The relative frequency of mediastinal involvement in NS patients is about 50%, whereas in MC patients, it is only about 25%. Of patients with mediastinal involvement, 58% have NS and 31% have MC types. The proportions are different (32% NS and 46% MC) in presentations without mediastinal involvement (table 8), difference significant at <0.001. The correlation between presentations with mediastinal involvement and sex is observed mainly for MC histologic type (table 9). Within this histologic type, presentation with mediastinal involvement is observed in 48% of women but in only 16% of

TABLE 8.—*Histologic type**

	LP	NS	MC	LD	Total No.
Mediastinum +	3%	58%	31%	8%	77
Mediastinum –	20%	32%	46%	2%	142
Total No.	30	91	90	8	219

*Significant at $P<0.001$.

men (table 9). It is also correlated with age. We studied the correlation between age and mediastinal involvement on the pooled series (retrospective and E.O.R.T.C.) to ensure a sufficiently large sample (table 10). Significantly more presentations with mediastinal involvement were found in patients between 15 and 50 years of age than in younger or older patients ($P<0.001$). The preponderance of incidence in the middle-aged group is much more pronounced in women than in men. All the data are explainable by 2 hypotheses: 1) The incidence of Hodgkin's disease is higher in men than in women; and 2) there is a high incidence of presentations with mediastinal involvement in women between 15 and 50 years.

TABLE 9.—*Incidence of presentation with or without mediastinal involvement; Stages I and II (E.O.R.T.C. series)*

		Mediastinum		Number of patients	
		−	+		
All histologic types	Male	72%	28%	168	$P<0.001$
	Female	51%	49%	109	
	Number of patients	175	102	277	
MC type	Male	84%	16%	74	$P<0.005$
	Female	52%	48%	25	
	Number of patients	75	24	99	
NS type	Male	57%	43%	49	NS*
	Female	45%	55%	44	
	Number of patients	48	45	93	

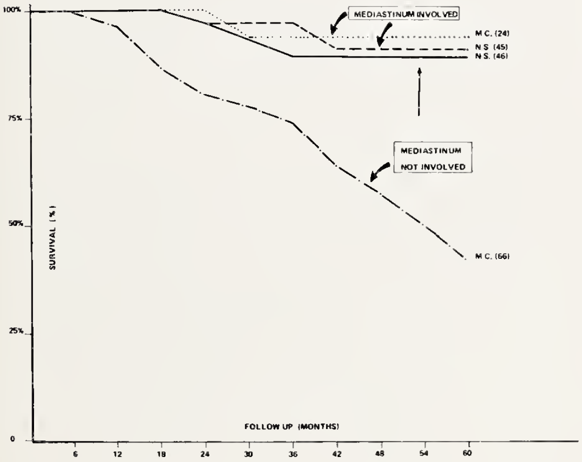
*Not significant.

TABLE 10.—*Hodgkin's disease—Stages I and II—relationship between sex, age, and presentation with mediastinal involvement—Retrospective E.O.R.T.C. series—Villejuif 1922–63*

Age (yr)	Female			Male			Total
	Without mediastinal involvement	With mediastinal involvement	Ratio: without mediastinal involvement/with mediastinal involvement	Without mediastinal involvement	With mediastinal involvement	Ratio: without mediastinal involvement/with mediastinal involvement	
<15	13(9%)	5(4%)	2.6	29(10%)	10(10%)	2.9	57
15–50	114(82%)	108(93%)	1	184(80%)	79(84%)	2.3	485
>50	12(9%)	3(3%)	4	22(9%)	5(6%)	4.4	42
Total	139 (100%)	116 (100%)	1.09	235 (100%)	94 (100%)	2.5	584

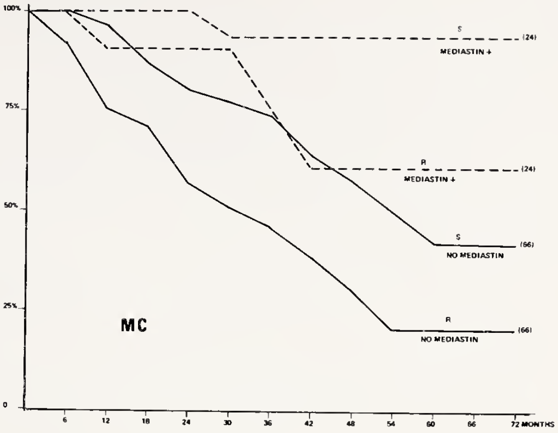
Influence on Prognosis of Histologic Type, Sex, Age, and Treatment for Patients With or Without Mediastinal Involvement

In patients with NS type, the prognosis is always good and is not significantly influenced by mediastinal involvement (text-fig. 6) or by sex. On the other hand, in patients with the MC type, the prognosis is strongly influenced by mediastinal involvement (text-figs. 6, 7), especially in men. In patients with mediastinal involvement, the prognosis is not influenced by sex and is only slightly influenced by the histologic type (text-fig. 6).



Text-figure 6.—Survival for patients with or without mediastinal involvement; various histologic types. E.O.R.T.C. series (clinical Stages I and II).

The association of long-term chemotherapy with radiotherapy only slightly improves the chance for survival of patients with mediastinal involvement (text-fig. 8), even for MC patients, but it is effective for patients without mediastinal involvement (text-fig. 9), in particular for MC patients (text-fig. 10). The previous discussion of long-term chemotherapy suggests that its main effect is to reduce the incidence of relapses in nonirradiated lymph-node areas. It seems, therefore, that the frequency of clinically undetectable, transdiaphragmatic, lymph-node involvement should be greater in patients without mediastinal involvement, especially in MC cases. The analysis of recurrences validates this prediction. Table 11 shows that the incidence of recurrence in a nonirradiated area on the same side of the diaphragm is similar for patients with and without mediastinal involvement. Conversely, the incidence of transdiaphragmatic recurrence in a nonirradiated lymph-node area is about 3 times greater in patients without mediastinal involvement (22/175 without; 5/102 with; significance at $P<0.02$). The incidence of transdiaphragmatic recurrence is low among patients with mediastinal presentation, even in MC cases (table 12).

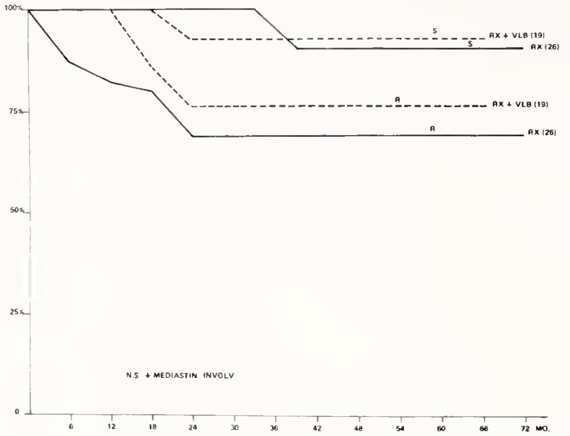


Text-figure 7.—Recurrence-free (R) and survival (S) data for patients with or without mediastinal involvement. MC type. E.O.R.T.C. series (clinical Stages I and II).

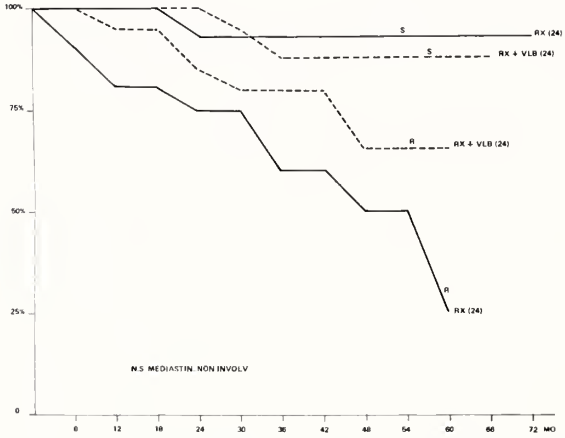
However, not all patients without mediastinal involvement exhibit the same tendency toward transdiaphragmatic lymph-node spread. The incidence of recurrence is high only for patients with MC type. Of patients with LP type, although 28 of 30 presented without mediastinal involvement, transdiaphragmatic lymph-node spread is extremely rare (1/28 patients). It is also small for patients with NS type (2 recurrences in 46 patients without mediastinal involvement).

Patterns of Presentation

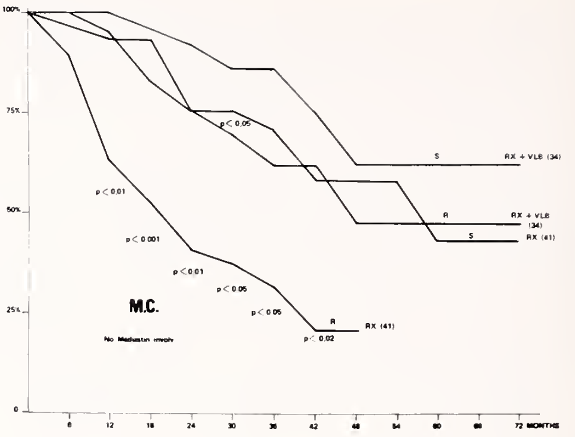
A few authors have discussed the possibility that Hodgkin's disease presents in more than 1 pattern. Kaplan (*1*) and Smithers (*13*) analyzed the spread of the disease and the mechanisms



TEXT-FIGURE 8.—Recurrence-free (R) and survival (S) data for patients with mediastinal involvement and NS type. No difference shown between patients treated by radiotherapy alone (RX) or by combination therapy with RX and vinblastine (VLB). E.O.R.T.C. series. Clinical Stages I and II.



TEXT-FIGURE 9.—Recurrence-free (R) and survival (S) data for patients without mediastinal involvement, NS type, clinical Stages I and II. Differences between patients treated by radiotherapy alone (RX) versus radiotherapy plus long-term chemotherapy with vinblastine (RX + VLB) are not statistically significant. E.O.R.T.C. series.



TEXT-FIGURE 10.—Recurrence-free (R) and survival (S) data for patients without mediastinal involvement, MC type, clinical Stages I and II. Differences between patients treated by radiotherapy alone (RX) versus combination of RX and vinblastine (VLB) are statistically significant. E.O.R.T.C. series.

involved in this diffusion process. Davidson and Clarke (*14*) proposed to divide patients with Hodgkin's disease into 2 groups. In the first, the lymphomatous process involves superficial lymph nodes—axillary and neck. Abnormalities of the pelvic or retroperitoneal glands also frequently coexist. This group includes patients with LP or MC type. In the second group, the lymphoreticular abnormality is predominantly in the mediastinum and the contiguous lymph glands in the lower neck and axillae. The para-aortic nodes are commonly involved. This group includes patients with NS or MC types. Davidson and Clarke showed that the average areas of the opacified nodes of abnormal lymphograms are significantly smaller in patients with mediastinal involvement. They also found that the peak age of onset is lower in this group and that the sex ratio is about 1:1. Finally they stated, but did not prove, that survival is longer in this second group of patients. It is evident that this model correlates in many ways with our data.

In 1971, Teillet et al. (*15, 16*) published 2 papers in which they distinguished 4 types of presentations at the time of diagnosis: *a*) high cervical localization, without mediastinal involvement; *b*) lower cervical and/or mediastinal localization, with about 85% mediastinal involvement; *c*) retroperitoneal or groin localization, without mediastinal involvement (half of these patients having the supraclavicular lymph nodes involved, the left more frequently than the right); and *d*) axillary localization which was associated in about half of the cases with mediastinal involvement.

These data suggested 4 separate patterns of

TABLE 11.—*Comparison of percentage of recurrences between the 2 groups defined by absence or presence of mediastinal involvement, according to site of recurrence (E.O.R.T.C. series)*

	Stages I and II		
	No mediastinal involvement	Mediastinal involvement	Total
Total patients at risk	175	102	277
Recurrence in irradiated area	6 (3.4%)	4 (3.9%)	10
Recurrence in nonirradiated area; same side of diaphragm	9 (5.1%)	6 (5.9%)	15
Recurrence in nonirradiated area; transdiaphragmatic	22 (12.6%)	5 (4.9%)	27
	$P<0.05$		
Recurrence, extranodal or systemic	19 (10.9%)	18 (17.6%)	37

TABLE 12.—*Incidence of transdiaphragmatic lymph-node recurrences in each subgroup (E.O.R.T.C. series)*

		LP	NS	MC	LD	Not sub-typed	Total
Mediastinal involvement	RX[*]	0/ 2[†]	3/26	0/10	1/4	0/16	4/58
	RX + VLB[‡]	0/ 0	0/19	1/14	0/2	0/ 8	1/43
	Total	0/ 2	3/45	1/24	1/6	0/24	5/101
No mediastinal involvement	RX	0/14	1/23	13/36	0/0	3/10	17/83
	RX + VLB	1/14	1/23	2/30	0/2	1/21	5/90
	Total	1/28	2/46	15/66	0/2	4/31	22/173

[*] Radiotherapy.

[†] Number of transdiaphragmatic recurrences/total number of patients at risk.

[‡] Vinblastine.

the disease: *a*) the upper cervical, which spreads to lower cervical lymph nodes and to axillary lymph nodes but not to the mediastinum and which is mostly of LP type; *b*) the axillary, which spreads to lower cervical or to the mediastinum; *c*) the mediastinal, which spreads to contiguous lymph nodes (supraclavicular, axillary, and para-aortic areas) and which is predominantly of NS type; and *d*) the retroperitoneal, which spreads mostly to the left supraclavicular lymph nodes and is often of MC type. They do not discuss the pattern of relapse and the natural history of the 4 types.

The E.O.R.T.C. data on initial presentation are slightly different. Of 296 E.O.R.T.C. patients, 29 had upper cervical lymph-node involvement (table 13). The mean age of these patients at diagnosis was 32 years. Only about one-third of them had LP type. In 18 patients, there was no other lymph-node involvement. In the remaining 11 presentations, other areas were involved; in 4 of these, the mediastinum was in-

TABLE 13.—*E.O.R.T.C. data on initial presentation*

Presentation	Number of patients	Sex ratio: male/female	Mean age at randomization (yr)	Number of patients subgrouped in histologic type	Percent of each histologic type				% survival at 4 years	% recurrence-free at 4 years	Number of recurrences		
											Total (%)	Unirradiated area	
					LP	NS	MC	LD				Same side (%)	Transdiaphragmatic (%)
Upper cervical (I + II)	29	2.2	32 ± 5.4	23	35% (8)	22% (5)	35% (8)	8% (2)	80.72	54.67	10 (35)	2 (7)	4 (14)
Upper cervical only (I)	18	2.6	33.4 ± 8	14	28.5% (4)	7% (1)	57.5% (8)	7% (1)	78.75	65.77	6 (33)	0	2 (11)
Mediastinum (I + II)	102	0.89	28.4 ± 2	77	2.5% (2)	57.5 (45)	30.5 (24)	9.5% (6)	83.42	57.70	33 (32.5)	6 (6)	5 (5)
Mediastinum only (I)	13	2.25	25.6 ± 5.8	12	8.5% (1)	66.5 (8)	25% (3)	0	100	57.35	3 (23)	1 (7.5)	1 (7.5)
Other presentations above diaphragm	151	2.06	29.5 ± 1.9	121	17.5% (20)	34% (42)	47% (58)	1.5% (1)	79	61.38	47 (31.5)	8 (5.5)	18 (12)
Below diaphragm	14	13	28.4 ± 6.6	13	23% (8)	15.5 (2)	61.5 (8)	0	84.61	31.01	6 (46)	0	3 (23)
Total	296												

volved. The incidence of mediastinal involvement is therefore low (4/29), but not negligible. On the other hand, it is much higher for patients with involvement of lower cervical lymph nodes (82/222).

A total of 151 patients had presentation without mediastinal involvement and without upper cervical lymph-node involvement. Of these, 47% were of MC type and 34% of NS type. The sex ratio was 2.05, and the mean age was 29.5 years.

Taking into account all these data, one can delineate at least 2 patterns of Hodgkin's disease. 1) The thoracic pattern. It involves the mediastinum and occurs mostly from puberty to menopause. Its frequency is equal in men and women. The incidence of para-aortic lymph-node relapse is low, but spread to the supraclavicular and axillary lymph nodes is frequent. Analysis of E.O.R.T.C. patients shows that the ESR is frequently elevated but does not carry a bad prognostic connotation. Both NS and MC types are observed; the first is more frequent, but the prognoses do not seem to differ. In this group the prognosis is not influenced by the sex of the patient. The usefulness of prophylactic irradiation of the para-aortic lymph nodes or of added chemotherapy is not obvious. Prognosis remains good even after recurrence. No statement can yet be made about the spleen. 2) Other supradiaphragmatic presentations. Probably they do not correspond to a single pattern. However, even if there are many patterns, it is still difficult to distinguish them. Those presentations are mostly observed in males. Analysis of the E.O.R.T.C. data shows that prophylactic irradiation of the para-aortic and iliac lymph nodes and/or association with chemotherapy are indicated, especially in males for whom the prognosis is worse than for females. Many of those presentations are probably of retroperitoneal origin especially for patients with involvement of supraclavicular fossa or of axillary lymph nodes. However, the disease in the abdomen may be concealed for many years, and it is difficult to recognize this pattern on a clinical basis. Analysis of transdiaphragmatic recurrences (table 10) suggests that the retroperitoneal pattern is predominantly, if not exclusively, of the MC type.

The need for distinguishing an upper cervical lymph-node presentation is not obvious from our data. In this presentation, involvement of the mediastinum is rare, but transdiaphragmatic recurrences are as frequent as in other supradiaphragmatic presentations without mediastinal involvement. The predominant histologic types are LP and MC.

The influences of sex and histology on the prognosis of Hodgkin's disease may be partly correlated with differences in pattern. Among patients with the MC type of disease, the clear-cut difference of prognosis between patients with or without mediastinal involvement must be stressed. Among those with the LP type, upper cervical lymph-node presentation is relatively frequent and mediastinal involvement rare. Prognosis is good and, whatever the presentation, transdiaphragmatic recurrence is rare.

The histologic type also seems to be correlated with the growth rate. NS is associated with a slow course and an orderly spread to contiguous areas. MC shows faster growth with a greater tendency to spread to areas where it was clinically undetectable at first examination, especially in presentations without mediastinal involvement.

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Survival and Relapse Data in Hodgkin's Disease: Freiburg im Breisgau, 1948–69 [1]

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SUMMARY—In the treatment of Hodgkin's disease, a focal dose of 4000 rads given in the course of 4 weeks consistently destroyed pathologic tissue in patients with lymphocytic predominance form. It was insufficient in some cases of other histologic forms and especially in the "lymphocytic depletion" type. In Stage I and especially Stage II, extended-field radiotherapy is superior to local therapy, but the diaphragm must not be considered as the lower limit of prophylactic therapy. With rare exceptions, prophylactic therapy should comprise at least the para-aortic lymph nodes and the spleen. In Stage III, total radiotherapy of all lymph nodes and the spleen is superior to all other therapeutic radiation methods. Extralymphatic progression can be prevented by an irradiation of the spleen or by splenectomy.—Natl Cancer Inst Monogr 36: 531–539, 1973.

IN HODGKIN'S DISEASE as in other tumors treatment must aim at a first remission of longstanding, since every relapse diminishes the prognosis for recovery. Of patients treated in our hospital from 1948 to 1960 by conventional local radiotherapy at 200 kv, only one patient among those in remission who is considered to be cured had a relapse in the meantime; the retreatment of this patient was finally successful. All the other patients with remission of at least 9 years' duration (*1*) were considered to be cured by the first treatment.

PATIENTS

To determine under what clinical, histopathologic, and therapeutic conditions remissions can be expected in Hodgkin's disease (relapses included), we selected a group of 96 untreated patients of Stages I–III. From 1964 to 1969, the Freiburg irradiation method (*2*), derived from Kaplan's method of treatment (*3*), was used on these patients with irradiation fields of various dimensions. We classified them as follows, according to Rye's classification: Stage I, 13 patients; Stage II, 37 patients; and Stage III, 46 patients.

METHODS AND RESULTS

In Stage I and II patients, the affected regions were locally irradiated. Alternately, the neighboring regions that were not yet involved were irradiated as a prophylactic meassure (*2*). For certain reasons we never irradiated beyond the diaphragm. However, we now recognize that, in cases in which the mediastinum and/or the left side of the neck had been affected, the thoracic duct can obviously serve as a bypass for spread of disease to the abdomen (*3*). Irradiation only to the diaphragm was therefore incomplete as a prophylaxis of neighboring regions in at least some of our cases.

Of the 50 patients classified as Stages I and II, 23 were subjected to local irradiation and 27 to more extensive irradiation down to the

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diaphragm. In the 23 patients in whom the affected regions only were irradiated, the disease reappeared in neighboring lymph nodes in 31%. In contrast there were 2 relapses, I involving the neighboring lymph-node region and 1 in a non-neighboring region, in the patients who had been irradiated down to the diaphragm (table I). In a patient in whom the lymph nodes of the left side of the neck and the mediastinum had been involved, the neighboring relapse was located para-aortally (text-fig. I). In another Stage I patient in whom supraclavicular lymph nodes had been involved, the non-neighboring relapse was also located para-aortally, in the iliacal region and probably in the spleen (text-fig. 2).

TABLE 1.—*Risk of progression dependent on radiotherapy (local and extended fields up to diaphragm) (Freiburg im Breisgau, 1964–69)*

Radiotherapy (^{60}Co)	Number	Progression after single radiotherapy									
				Lymphoma							
		Total		Local		Contiguous		Noncontiguous		Organs	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Local fields	23	11	49	3*	13	7*	31	2	9	2*	9
Extended fields	27	7	26	4	15	1†	4	1‡	4	1	4
	50	18		7		8		3		3§	

*Relapses in different regions.

†Mediastinal involvement with progression on para-aortic lymph nodes.

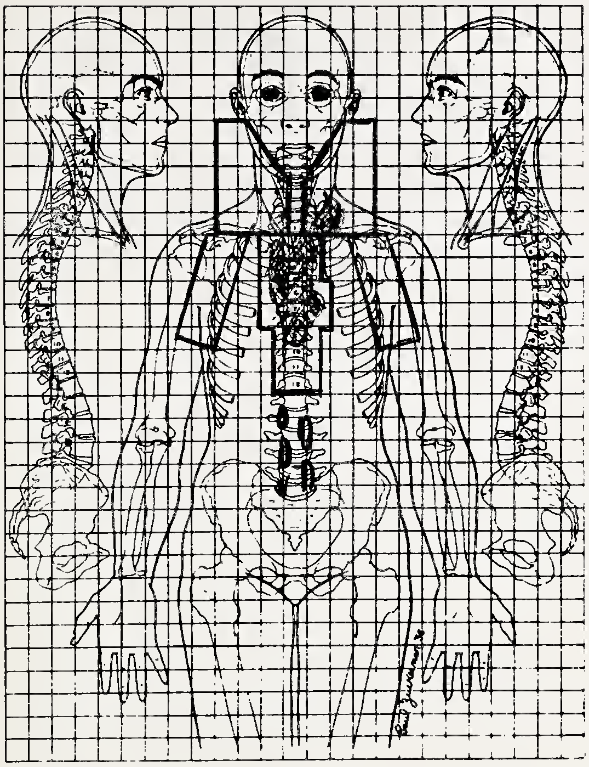
‡Left supraclavicular involvement with progression on para-aortic lymph nodes.

§All relapses in contiguous organs.

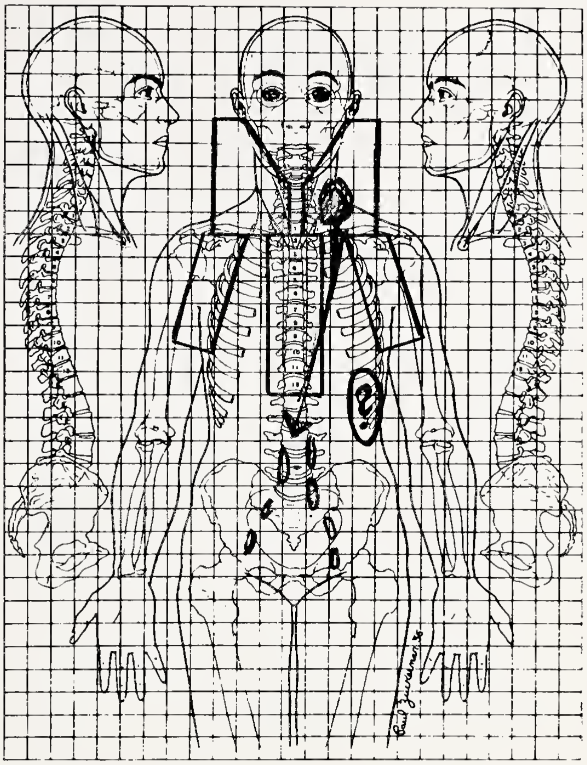
Relapses would have been avoided in both cases if, in the prophylactic radiotherapy, two factors had been considered: 1) that the diaphragm does not stop the spread of the disease and 2) that spread from the supraclavicular lymph nodes can proceed directly via the thoracic duct, thus bypassing the mediastinum. As compared with the relatively large number of neighboring relapses in locally irradiated patients, in both groups only a few relapses occurred in non-neighboring regions and organs.

The reappearance and the progression of the disease in the Stage I and II patients depended on the therapy practiced, as was shown above. Moreover, both were a function of the number of lymph-node regions affected and of the presence of clinical signs of activity in B form of the disease. The higher the number of affected lymph-node regions in Stages I or II, the greater is the risk that the disease will progress beyond the irradiation fields. This rule applies to spreading in the lymphatic system as well as to extralymphatic extensions. However, we have observed extension to nonlymphatic organs only in those patients in whom at least 4 regions have been affected and never in those patients in whom 1–3 regions have been affected. The rule holds if local relapses attributed directly to the radiation dose are not considered. The rule applies to local irradiation and to the extended field of irradiation though, in the latter instance, the number of relapses is lower (table 2). Tubiana and co-workers (*4*) came to similar conclusions in their report on the prognostic factors in Hodgkin's disease.

The clinical B-symptoms of progress, where therapy was undertaken of the disease, influenced the rate of neighboring relapses. Relapses in neighboring lymph nodes occurred in 31% of the patients in Stage II-B and in only 16% of the patients in Stage II-A. There were no B cases among the patients of Stage I. The number of relapses in non-neighboring lymph nodes or in other organs was small and almost



TEXT-FIGURE 1.—Hodgkin's disease Stage II-B (Rye), nodular sclerosis. Involvement of left supraclavicular and mediastinal lymph nodes. Progression of the disease in the para-aortal lymph nodes 15 months after radiation therapy of all lymph-node regions above diaphragm. Second radiation therapy was followed by recovery lasting for 3 years to present.



TEXT-FIGURE 2.—Hodgkin's disease Stage I-A (Rye), nodular sclerosis. Involvement of left supraclavicular lymph nodes. Progression of the disease in the para-aortal and left iliacal lymph nodes and enlargement of the spleen 9 months after radiation therapy of all lymph-node regions above diaphragm. Second radiation therapy of all abdominal lymph nodes including spleen was followed by recovery lasting for 3 years to present.

TABLE 2.—*Hodgkin's disease, Stage I and II (Rye): risk of progression after initial radiotherapy (local and extended fields) as affected by number of involved lymph-node regions*

Number of involved lymph-node regions	Relapses and progression			
	Total		Total without local relapses	
	n*	Percent	n*	Percent
1	2/13	15	2/13	15
2, 3, and 4	11/30	36.5	8/30	27
5	5/7	72	4/7	57
	18/50		14/50	

*Number of relapses/No. of patients at risk.

equal in both clinical groups A and B with both forms of therapy (table 3).

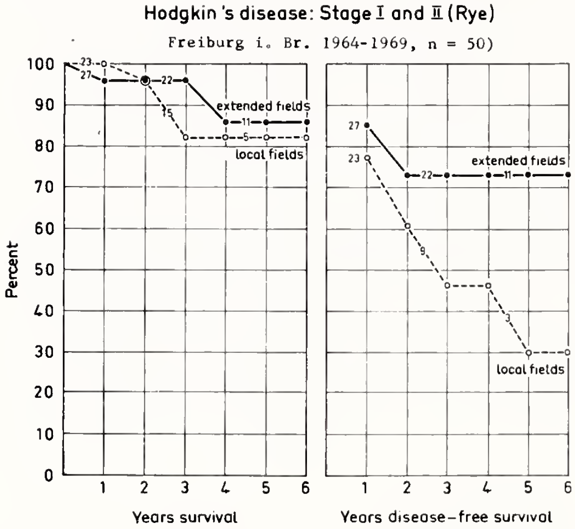
The greater number of relapses occurring after local radiotherapy as compared with the extended field irradiation is, as expected, less apparent from the survival curve covering an observation period of only up to 7 years than from the curve of relapse-free survival (text-fig. 3).

In the group in which the 5 supradiaphragmatic regions (both sides of the neck, both armpits, and the mediastinum) were affected, radiotherapy down to the diaphragm was equivalent to local treatment. In this small group of 7 patients, we recorded the lowest survival curve; moreover, all patients had relapses in the course of 5 years (text-fig. 4).

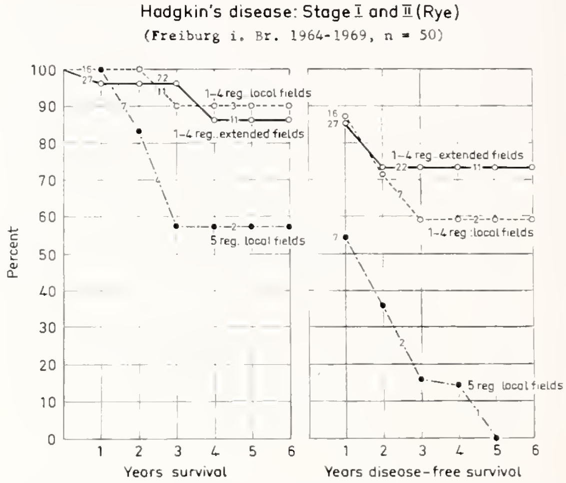
The 46 patients in Stage III were treated in

TABLE 3.—*Hodgkin's disease, Stage I and II (Rye): Risk of progression after initial radiotherapy (local and extended fields) related to clinical symptoms A and B (Freiburg im Breisgau, 1964–69)*

		Relapses and progression									
				Lymphoma							
		Total		Local		Contiguous		Noncontiguous		Organs	
Stage	Number	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
I-A	13	2	15	1	8			1	8		
I-B	0										
II-A	24	8	33.3	4	16	4	16	1	4	2	8
II-B	13	8	61	2	15	4	31	1	8	1	8



TEXT-FIGURE 3.—Hodgkin's disease, Stages I and II (Rye), primary untreated cases. Actuarial analysis of survival and continuous disease-free survival subsequent to radiation therapy with local fields and extended fields to the diaphragm (Freiburg im Breisgau, 1964–69).



TEXT-FIGURE 4.—Hodgkin's disease, Stages I and II (Rye), primary untreated cases. Actuarial analysis of survival and continuous disease-free survival subsequent to radiation therapy and subsequent to the number of involved lymph-node regions (Freiburg im Breisgau, 1964–69).

different ways during the observation period from 1964 to 1969. In 14, 5 without and 9 with B clinical symptoms, total-nodal and lienal radiotherapy was given; in 23, the lymph nodes except the spleen were irradiated. In 9 patients, an extended local irradiation was performed, which included the spleen in 2 of the patients (table 4).

All 5 patients in group III-A who received a total-nodal irradiation including the spleen have been to the present time in a state of complete remission. The 9 patients in group III-B who were treated in the same manner suffered (in addition to the local relapses to be discussed below) a lymph-node relapse in a prophylactically irradiated region, a lienal relapse in an obviously normal spleen, and extension of the disease to extranodal organs in 2 cases (22%). Of the 23 patients in Stages III-A and III-B to whom we gave total-nodal treatment (but no irradiation of the obviously normal spleen),

TABLE 4.—*Hodgkin's disease, Stage III (Rye): Risk of progression related to form of radiotherapy (total-nodal and extended fields) with and without radiotherapy of spleen (Freiburg im Breisgau, 1964–69, number = 46)*

Radiotherapy technique		Stage	Number of patients		Relapses and progression									
							Lymphoma							
			Total	With positive spleen in clinical or laparoscopy investigation	Total		Local		Other		Spleen		Organs	
					Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Total-nodal	including spleen	III-A	5	1	0									
		III-B	9	3	5	55	3	33	1	11	1*	11	2	22
	without spleen	III-A and III-B	23	0	15	65	7	31	0	0	5†	22	11	48
Extended fields without spleen‡		III-A and III-B	9	2	6	67	1	11	3	33	1	11	3	33
Total			46	6	26		11		4		7		16	

*Coincident with liver involvement.

†3 of 5 coincident with extralymphatic organ involvement.

‡2 of this group with radiation therapy of the spleen.

5 patients (22%) had lienal relapses, and 11 patients (48%) had disease spread to extranodal organs. In the group of 9 patients in whom the spleen and total lymph-node regions had not been irradiated systematically, 6 had relapses in the lymph nodes, spleen, and extralymphatic organs.

While in Stages I and II, a relationship existed between the frequency of relapse and the number of lymph-node regions affected, such a relationship was not observed in Stage III. This finding is consistent with the data published by Tubiana and co-workers (D). The percentage of relapses was equal in patients in whom 2–5 or 6–8 regions were involved. In both groups of Stage III, the frequency of relapses depended mainly on the treatment of the spleen; if the latter was not included in the radiotherapy treatment, the frequency of relapses almost doubled in both groups (table 5).

TABLE 5.—*Hodgkin's disease, Stage III (Rye): Risk of progression after initial radiotherapy with and without radiation of spleen (Freiburg im Breisgau, 1964–69, number = 46)*

Number of involved lymph-node regions	Relapses dependent on radiotherapy technique			
	Total-nodal plus spleen		Total-nodal (23)† and extended fields (9)† without spleen	
	n*	Percent	n*	Percent
2–5	2/6	33	7/10	70
6–8	3/8	37	14/22	64

*Number of relapses/No. of patients at risk.
†Numbers in parentheses are number of patients.

In analyzing the correlation between relapses in Stage III and a given radiotherapy, we came to the following conclusions: *a*) In Stage III patients, if lymph nodes and the spleen have not been irradiated, the disease will progress in that particular region in a high percentage of the cases; and *b*) if the spleen, whether clinically affected or not, has not been irradiated, the relapse rate in extralymphatic organs will increase from 14 to 44%. These figures show a surplus of 30% of organ relapses in the group without splenic irradiation as compared to the group with splenic irradiation (table 6).

These findings suggest that the spleen is an early source of generalized metastasis even if it presents no clinical signs of the disease. Moreover, the findings coincide with the data of the group from Stanford University School of Medicine, who reported not only that the spleen is the most frequently involved abdominal organ in Hodgkin's disease (*5*), but also that the involvement of the liver is related to that of the spleen (*3*). These findings also correspond to the observations of Rappaport et al. (*6*), who observed that vascular invasion is 3 times as frequent in the spleen (3/13=23%) as in lymph nodes (4/49=8%). Our findings suggest that a therapeutic prophylaxis in Hodgkin's disease, that does not include the spleen, should be considered incomplete.

In all our patients only in local relapses was there a correlation between the appearance of relapses and the histologic form. Of the 78 patients who were histologically classified, 10 had local relapses. After a uniform, focal dose of 4000 rads applied in the course of 4–5 weeks, we recorded 33.3% local relapses in the lymphocytic depletion group and approximately 10% in the mixed-cellularity and nodular-sclerosis groups. In the lymphocytic predominance form, no local relapse occurred (table 7).

Because it was associated with a rather low rate of local relapses with conventional 200 kv radiotherapy, 4000 rads has been considered the necessary tumoricidal dose level in Hodgkin's disease (*7, 8*). However, our findings suggest that the relative biologic efficiency of cobalt irradiation in lymphogranulomatosis is inferior to that of 200 kv X-rays, and that the focal destruction dose is also related to the histologic form of the disease.

The rates of survival and relapse-free survival

TABLE 6.—*Hodgkin's disease, Stage III: Risk of extralymphatic progression related to radiotherapy of spleen (Freiburg im Breisgau, 1948–69, number = 46)*

Radiotherapy technique		Extralymphatic progression	
		n*	Percent
Total-nodal (14)	plus spleen	2/14	14
Total-nodal (23) and extended fields (9)†	without spleen	14/32	44
Difference			30

*Number of extralymphatic progression/No. of patients at risk.
†2 of this group with radiation therapy of spleen.

TABLE 7.—*Hodgkin's disease, Stage I–III: Local relapses subsequent to radiotherapy with 4000 rads/4–5 weeks (Freiburg im Breisgau, 1964–69)*

Histology	Relapses	
	n*	Percent
Lymphocytic predominance	0/13	0
Nodular sclerosis	3/27	11
Mixed cellularity	2/23	9
Lymphocytic depletion	5/15	33.3
	10/78	13

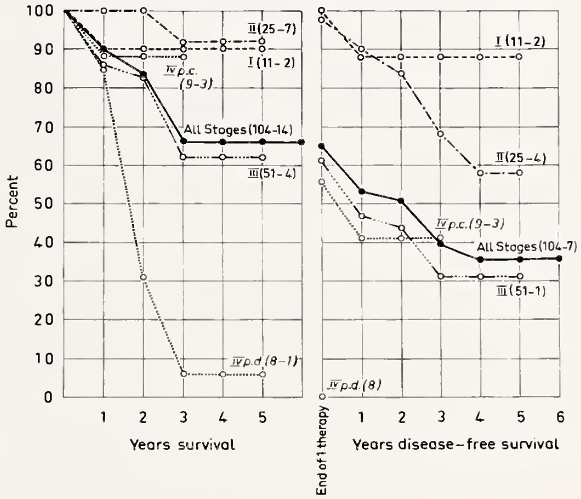
*Number of relapses per person, not per irradiation area/No. of patients at risk.

we have recorded since we put a cobalt-60 (^{60}Co) source into operation in 1964 are as follows: In Stage I, the 5-year survival rate is 90%, and the relapse-free survival rate is 88%. In Stage II, the corresponding figures are 92 and 57%; while in Stage III, they are 63 and 31%. For Stage IV per continuitatem, we have recorded 3-year rates of 88 and 41% and for Stage IV per dissemination rates of 6 and 0% (*9*). The 5-year survival rate for all stages in the years 1964–69 was 66%, while the relapse-free survival rate was 36% (text-fig. 5).

DISCUSSION AND THERAPEUTIC IMPLICATIONS

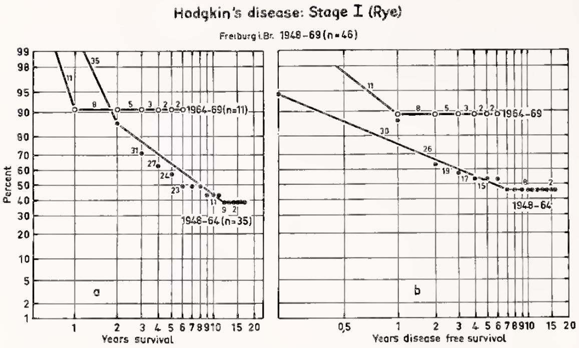
These results of a therapeutic policy that is not completely optimal as indicated by present controls are nonetheless significantly superior to those of the conventional X-ray therapy practiced from 1948 to 1964. In Stage I the 5-year survival rate was increased from 57 to 90%, while the rate of relapse-free survival rose from 54 to 88% (text-fig. 6).

Hodgkin's disease: all Stages
(Freiburg i.Br.1964–1969 primary untreated cases; n=104)



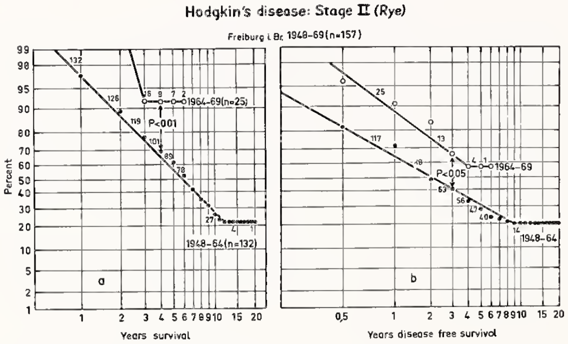
TEXT-FIGURE 5.—Hodgkin's disease, primary untreated cases, all stages. Actuarial analysis of survival and continously disease-free survival (Freiburg im Breisgau, 1964–69).

In Stage II, the 5-year survival rate increased from 61 to 92%, while the rate of relapse-free survival rose from 29 to 58% (text-fig. 7). In Stage III, the survival rate increased from 23 to 63%, and the rate of relapse-free survival from 2.7 to 37% (text-fig. 8). Since the number of cases was too small in Stages IV per continuitatem and per dissemination, we made no comparison. However, the results showed improve-



Text-figure 6.—Hodgkin's disease, Stage I (Rye), primary untreated cases. Actuarial analysis of survival and continuously disease-free survival subsequent to radiation therapy. 200 kv X-ray therapy, mainly local fields, in the period from 1948 to 1964. ^{60}Co therapy, mainly extended fields in the period from 1964 to 1969.

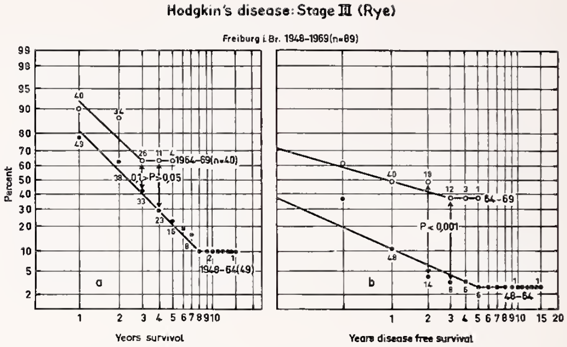
ment in these stages too. The total 5-year survival rate was at 42% before 1964 and has risen to 66%. The corresponding figures are 19% and 36% for the rate of relapse-free survival (text-fig. 9).



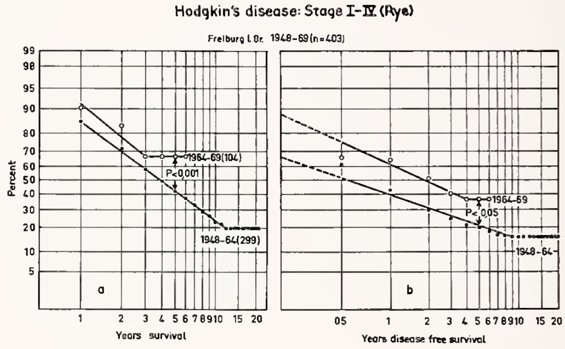
Text-figure 7.—Hodgkin's disease, Stage II (Rye), primary untreated cases. Actuarial analysis of survival and continuously disease-free survival subsequent to radiation therapy. 200 kv X-ray therapy mainly local fields in the period from 1948 to 1964. ^{60}Co therapy, mainly extended fields in the period from 1964 to 1969.

In 1971 Frei and Gehan (*10*) published a compilation of data in which they attempted to prove that a relapse-free survival of 4 years is tantamount to an 80% chance of cure. Applying their figures to our few cases, we can estimate a projected cure rate of 70% in Stage I, of 45% in Stage II, and of 30% in Stage III.

In analyzing the successes and failures of the radiotherapy program carried out primarily in Freiburg between 1964 and 1969, we have come to several conclusions: 1) Under the conditions of high-voltage radiotherapy, a focal dose of 4000 rads in the course of 4 weeks will consistently destroy the pathologic tissue only in the lymphocytic predominance form. It is insufficient in some cases of other histologic forms and especially in the lymphocytic depletion form. 2) In Stage I and especially in Stage II extended-field therapy is superior to local therapy, but the diaphragm must not be considered as the lower limit of prophylactic therapy. With rare exceptions, prophylactic irradiation should comprise at least the para-aortic lymph nodes and the



Text-figure 8.—Hodgkin's disease, Stage III (Rye), primary untreated cases. Actuarial analysis of survival and continuously disease-free survival subsequent to radiation therapy. 200 kv X-ray therapy, mainly local fields in the period from 1948 to 1964. ^{60}Co therapy, mainly extended fields in the period from 1964 to 1969.



Text-figure 9.—Hodgkin's disease, primary untreated cases, all stages. Actuarial analysis of survival and continuously disease-free survival subsequent to radiation therapy. 200 kv X-ray therapy, mainly local fields in the period from 1948 to 1964. ^{60}Co therapy, mainly extended fields in the period from 1964 to 1969.

spleen. This conclusion coincides with the findings of Johnson et al. (*11*) and of Kaplan (*3*). 3) In Stage III, total radiotherapy of the lymph nodes and the spleen is superior to all other therapeutic radiation methods. Extralymphatic progression can be prevented by irradiation of the spleen or by splenectomy.

It is hoped that advances in radiotherapy, as a result of the above findings, will promote the achievement of even better results in the near future. Because the chance of recovery lessens with each recurrence of the disease, it should be the goal of every radiation treatment to include all risk zones within the irradiated fields from the start of therapy.

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Information Storage and Retrieval System for the Study of Hodgkin's Disease[1,2,3]

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SUMMARY—An elaborate and innovative computer-based information storage and retrieval system developed at Stanford University Medical Center for clinical research on Hodgkin's disease is described. The system allows detailed analysis of multiple patient parameters and is sufficiently flexible to permit adaption to and entry of variables not foreseen at the time of initiation of data storage.—Natl Cancer Inst Monogr 36: 541–553, 1973.

THE NEED for detailed analysis of a large and complex series of clinical trials involving patients with Hodgkin's disease prompted development of a specialized information storage and retrieval system, which is described here in the hope that it may prove useful to other centers in which such studies are in progress. As currently constituted, this system has 3 goals: 1) the input of a data base sufficiently detailed to permit a wide spectrum of multifactorial analyses to be performed with the appropriate retrieval programs; 2) the capability to modify, enhance, or delete information from the data base; and 3) a program which facilitates both general and detailed retrieval of patient data. The computer utilized for the system is an IBM 360 model 50, access to which is obtained by remote typewriter (IBM 2741) terminal. The computer language used is a modification of PL-1 (PL/ACME).

DATA BASE

The data base for this system is divided into the following 3 files: 1) pretreatment diagnostic and staging data, 2) treatment data, and 3) follow-up data. This organization is well adapted to the usual chronologic sequence in which patient data are recorded. Each computer file consists of information abstracted from patient records, recorded on forms, and then accessed to the specific file.

The pretreatment file contains all pertinent information derived after an extensive diagnostic evaluation including history, physical examination, radiographic studies, laboratory data, and histopathologic correlations. The clinical stage (CS) and pathologic stage (PS) designations used conform to the Ann Arbor classification (*1*).

The treatment file contains information describing each course of treatment the patient has received. For each course of radiotherapy, the time, dose, and volume relationship are detailed as well as the dates of treatment, in-

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[2] Supported by Public Health Service grant CA05838 from the National Cancer Institute.

[3] Computer support for this research was obtained from the ACME (Advanced Computer for Medical Research) Facility at the Stanford Medical Center. ACME is supported by Public Health Service grant RR00311 from the Biotechnology Resources Branch of the National Institutes of Health. The assistance of Mrs. Polly Butterfield with programming is gratefully acknowledged.

terruptions, complications, and the peripheral blood counts. Isotopic therapy and chemotherapy are detailed in similar fashion.

The follow-up file contains information obtained after the patient's initial treatment. It gives details on the routine physical examination, radiographic studies, and laboratory tests used to determine the disease status, types of treatment, complications of disease, and complications of treatment.

SAMPLE CASE HISTORY

To facilitate description of the system, the following fictitious case history is provided. Details of this case history have been entered on the various forms used for input into the 3 files described.

John Swenson, a 32-year-old male, first noted a left axillary mass in November 1967. After a course of antibiotics failed to diminish the size of the mass, a biopsy was performed on January 29, 1968, which disclosed nodular-sclerosing Hodgkin's disease. The patient was entirely asymptomatic. On physical examination, an indurated scar in the left axilla was noted, but there was no palpable lymphadenopathy or hepatosplenomegaly. Liver function tests, bone marrow biopsy, and radiographic studies, including lymphangiography, failed to show any disease other than in the left axilla. Exploratory laparotomy was not performed. The patient was officially staged as CS-IA_-, PS-IA_{M-} was randomly allocated to treatment option A (involved-field radiotherapy only) under protocol L-1, and received 4400 rads to the left axilla in 26 days.

From the completion of treatment in March 1968, the patient has been followed regularly in the lymphoma clinic and thought to show no evidence of disease until July 1970. On repeat lymphangiogram, an abnormal para-aortic lymph node was noted. He was asymptomatic, and physical examination disclosed no lymphadenopathy or hepatosplenomegaly. An exploratory laparotomy, splenectomy, and biopsy of the liver, para-aortic lymph nodes, and bone marrow were performed on July 14, 1970. Hodgkin's disease was demonstrated in the spleen and splenic hilar nodes. The biopsy of para-aortic lymph nodes (which did not include the abnormal node seen on the lymphangiogram) revealed lymphangiogram effect only. The liver and bone marrow biopsies were negative.

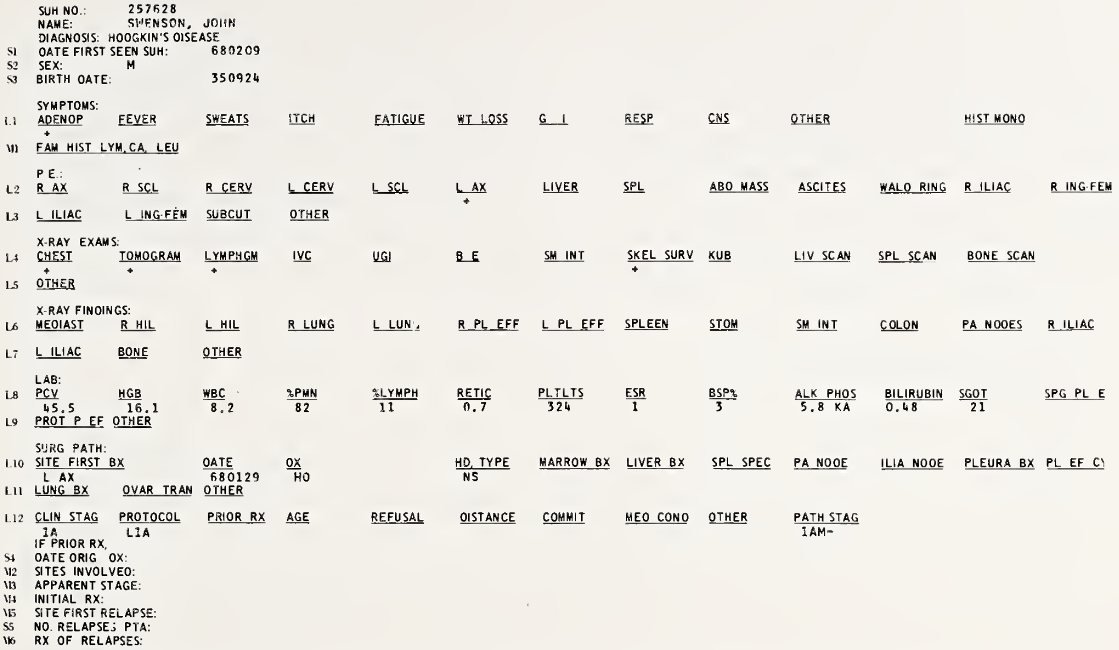
The patient was then treated with total-nodal irradiation, with a lead block shielding the previously treated left axilla, followed by 6 cycles of quadruple drug therapy—a combination of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP). He tolerated the treatment well, except for the development of a mild Lhermitte's syndrome that persisted for only 3 months.

The patient has remained asymptomatic, and physical examination has disclosed no evidence of disease. Follow-up radiographic studies and liver function tests have remained negative. He is therefore apparently free of disease, 1 year after retreatment with total-nodal radiotherapy and 6 months after MOPP chemotherapy.

DATA INPUT

The pretreatment diagnostic and staging data on this patient have been entered in the data input form for file 1 (text-fig. 1). The entries at the upper left include the patient's name, hospital record number, sex, date of birth, and date of admission (entered as year, month, day). The fact that the patient had noted a left axillary mass is denoted by a plus (+) symbol on line L1 under "ADENOP." Other symptoms have been left blank, since they did not occur in this case. The only significant physical findings, entered on line L2, again relate to the left axilla. Line L4 records the X-ray examinations; in this instance, these were conventional chest roentgenograms and tomograms, lymphangiogram, and skeletal survey. Line L6, on which positive X-ray findings are indicated, has been left blank because all these X-ray studies were normal initially. Relevant laboratory values are indicated in actual numerals in line L8. The site and date of the first biopsy are entered on line L10, together with the diagnosis, the histologic type (if Hodgkin's disease), and the results of any other biopsies performed during initial diagnostic evaluation. Since the patient did not have an exploratory laparotomy at the first diagnostic evaluation, no entries were made under "LIVER BX," "SPL SPEC," or "PA NODE," etc. To complete the data input for this file, the clinical stage, protocol treatment assignment, and pathologic stage are given on line L12.

The initial course of radiotherapy, which was limited to the left axillary region, is recorded on the treatment-data input form (text-fig. 2). The total dose in rads is listed under "L AX" followed by the number of treatments, the overall elapsed treatment time in days, the dates of first and last treatment (year, month, day), the megavoltage treatment machine used, the lowest white blood cell count, lowest packed cell volume or hemoglobin (in this instance "H" refers to the hemoglobin level of 13.7 g/100 ml), and the lowest platelet count in thousands. Lines L7 and L8 are used to record interruptions of treat-



```
     SUH NO.:        257628
     NAME:           SWENSON,  JOHN
     DIAGNOSIS:  HODGKIN'S DISEASE
S1   DATE FIRST SEEN SUH:      680209
S2   SEX:            M
S3   BIRTH DATE:               350924

     SYMPTOMS:
L1   ADENOP    FEVER     SWEATS    ITCH      FATIGUE   WT LOSS   G  I      RESP      CNS       OTHER               HIST MONO
      +
M1   FAM HIST LYM,CA, LEU

     P E.:
L2   R AX      R SCL     R CERV    L CERV    L SCL     L AX      LIVER     SPL       ABD MASS  ASCITES   WALD RING R ILIAC   R ING-FEM
                                                        +
L3   L ILIAC   L ING-FEM SUBCUT    OTHER

     X-RAY  EXAMS:
L4   CHEST     TOMOGRAM  LYMPHGM   IVC       UGI       B E       SM INT    SKEL SURV KUB       LIV SCAN  SPL SCAN  BONE SCAN
      +         +         +                                                 +
L5   OTHER

     X-RAY FINDINGS:
L6   MEDIAST   R HIL     L HIL     R LUNG    L LUNG    R PL EFF  L PL EFF  SPLEEN    STOM      SM INT    COLON     PA NODES  R ILIAC

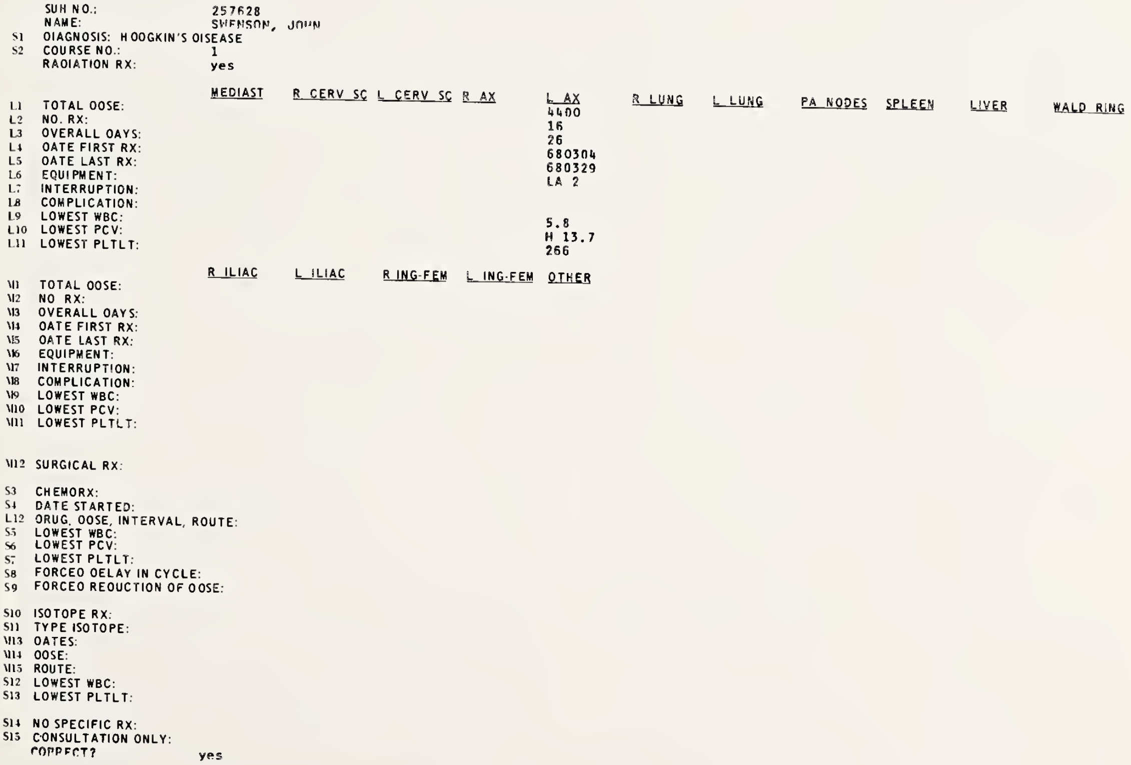
L7   L ILIAC   BONE      OTHER

     LAB:
L8   PCV       HGB       WBC       %PMN      %LYMPH    RETIC     PLTLTS    ESR       BSP%      ALK PHOS  BILIRUBIN SGOT      SPG PL E
      45.5      16.1      8.2       82        11        0.7       324       1         3         5.8 KA    0.48       21
L9   PROT P EF OTHER

     SURG PATH:
L10  SITE FIRST BX       DATE      DX                  HD, TYPE  MARROW BX LIVER BX  SPL SPEC  PA NODE   ILIA NODE PLEURA BX PL EF C
      L AX                680129    HO                  NS
L11  LUNG BX   OVAR TRAN OTHER

L12  CLIN STAG PROTOCOL  PRIOR RX  AGE       REFUSAL   DISTANCE  COMMIT    MED COND  OTHER     PATH STAG
      1A        L1A                                                                             1AM-
     IF PRIOR RX,
S4   DATE ORIG  DX:
M2   SITES INVOLVED:
M3   APPARENT STAGE:
M4   INITIAL  RX:
M5   SITE FIRST RELAPSE:
S5   NO. RELAPSES PTA:
M6   RX OF RELAPSES:
```

TEXT-FIGURE 1.—Computer data input form for pretreatment diagnostic and staging data, with entries for the fictitious patient described in the text.



```
      SUH NO.:             257628
      NAME:                SWENSON,  JOHN
S1    DIAGNOSIS:  HODGKIN'S DISEASE
S2    COURSE NO.:          1
      RADIATION RX:        yes

                           MEDIAST   R CERV SC L CERV SC R AX      L AX      R LUNG    L LUNG    PA NODES  SPLEEN    LIVER     WALD RING
L1    TOTAL DOSE:                                                  4400
L2    NO. RX:                                                      16
L3    OVERALL DAYS:                                                26
L4    DATE FIRST RX:                                               680304
L5    DATE LAST RX:                                                680329
L6    EQUIPMENT:                                                   LA 2
L7    INTERRUPTION:
L8    COMPLICATION:
L9    LOWEST WBC:                                                  5.8
L10   LOWEST PCV:                                                  H 13.7
L11   LOWEST PLTLT:                                                266

                           R ILIAC   L ILIAC   R ING-FEM L ING-FEM OTHER
M1    TOTAL DOSE:
M2    NO  RX:
M3    OVERALL DAYS:
M4    DATE FIRST RX:
M5    DATE LAST RX:
M6    EQUIPMENT:
M7    INTERRUPTION:
M8    COMPLICATION:
M9    LOWEST WBC:
M10   LOWEST PCV:
M11   LOWEST PLTLT:


M12   SURGICAL RX:

S3    CHEMORX:
S4    DATE STARTED:
L12   DRUG, DOSE, INTERVAL, ROUTE:
S5    LOWEST WBC:
S6    LOWEST PCV:
S7    LOWEST PLTLT:
S8    FORCED DELAY IN CYCLE:
S9    FORCED REDUCTION OF DOSE:

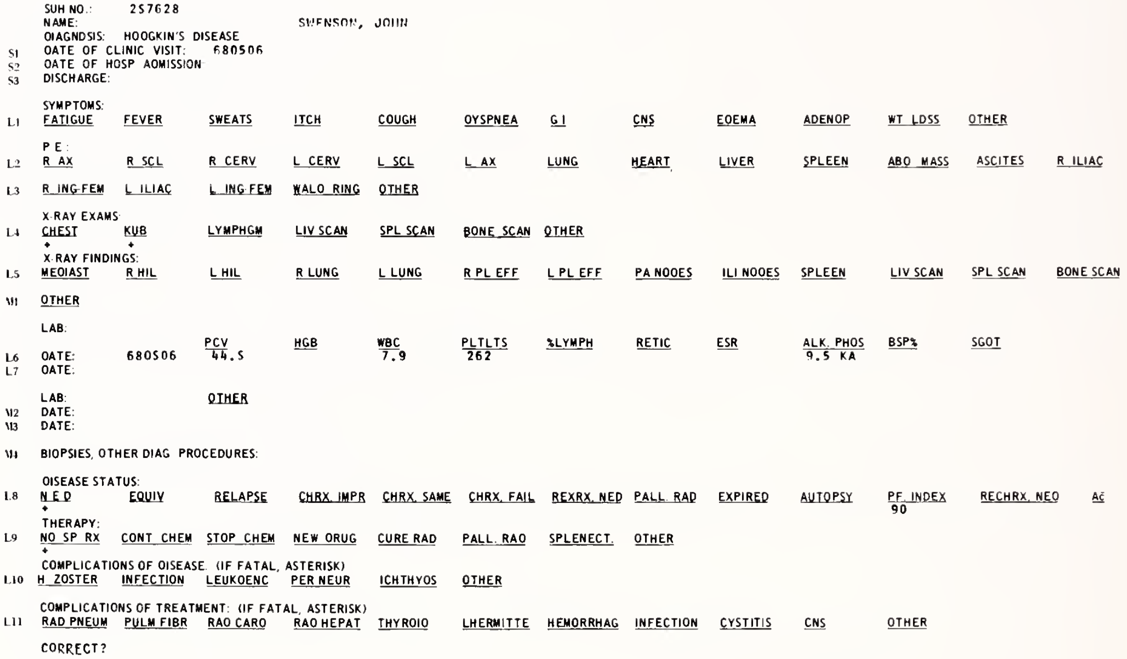
S10   ISOTOPE RX:
S11   TYPE ISOTOPE:
M13   DATES:
M14   DOSE:
M15   ROUTE:
S12   LOWEST WBC:
S13   LOWEST PLTLT:

S14   NO SPECIFIC RX:
S15   CONSULTATION ONLY:
      CORRECT?             yes
```

TEXT-FIGURE 2.—Data input form for the first course of radiotherapy in the sample case, involving local treatment to the left axilla.

ment or significant complications during treatment, neither of which occurred in this case.

The patient's status at the first post-treatment follow-up examination of May 6, 1968, is entered on the first follow-up data input form (text-fig. 3). On this occasion, the patient had no symptoms and physical examination was negative. Chest and abdominal ("KUB") radiographic examinations were performed and gave normal results. The relevant laboratory data on this occasion are entered on line L6, where the serum alkaline phosphatase data refer to King-Armstrong ("KA") units. On line L8, the examining physician concluded that the patient had no evidence of disease ("NED"), and therefore a plus (+) sign is entered under this heading. The Karnofsky performance index was rated at 90%.



```
      SUH NO.:     257628
      NAME:                                    SWENSON, JOHN
      OIAGNDSIS:  HOOGKIN'S DISEASE
S1    OATE OF CLINIC VISIT:    680506
S2    OATE OF HOSP AOMISSION
S3    DISCHARGE:

      SYMPTOMS:
L1    FATIGUE   FEVER     SWEATS    ITCH      COUGH     OYSPNEA   G I       CNS       EOEMA     ADENOP    WT LDSS   OTHER

      P E :
L2    R AX      R SCL     R CERV    L CERV    L SCL     L AX      LUNG      HEART     LIVER     SPLEEN    ABO MASS  ASCITES   R ILIAC

L3    R ING-FEM L ILIAC   L ING-FEM WALO RING OTHER

      X-RAY EXAMS
L4    CHEST     KUB       LYMPHGM   LIV SCAN  SPL SCAN  BONE SCAN OTHER
      +         +
      X-RAY FINDINGS:
L5    MEOIAST   R HIL     L HIL     R LUNG    L LUNG    R PL EFF  L PL EFF  PA NOOES  ILI NOOES SPLEEN    LIV SCAN  SPL SCAN  BONE SCAN

M1    OTHER

      LAB:
                          PCV       HGB       WBC       PLTLTS    %LYMPH    RETIC     ESR       ALK. PHOS BSP%      SGOT
L6    OATE:     680506    44.5                7.9       262                                     9.5 KA
L7    OATE:

      LAB:                OTHER
M2    DATE:
M3    DATE:

M4    BIOPSIES, OTHER DIAG PROCEDURES:

      OISEASE STATUS:
L8    N E D     EQUIV     RELAPSE   CHRX. IMPR CHRX. SAME CHRX. FAIL REXRX. NED PALL. RAD EXPIRED AUTOPSY PF. INDEX RECHRX. NEO AC̄
      +                                                                                            90
      THERAPY:
L9    NO SP RX  CONT CHEM STOP CHEM NEW ORUG  CURE RAD  PALL. RAO SPLENECT. OTHER
      +
      COMPLICATIONS OF OISEASE. (IF FATAL, ASTERISK)
L10   H ZOSTER  INFECTION LEUKOENC  PER NEUR  ICHTHYOS  OTHER

      COMPLICATIONS OF TREATMENT: (IF FATAL, ASTERISK)
L11   RAD PNEUM PULM FIBR RAO CARO  RAO HEPAT THYROIO   LHERMITTE HEMORRHAG INFECTION CYSTITIS  CNS       OTHER

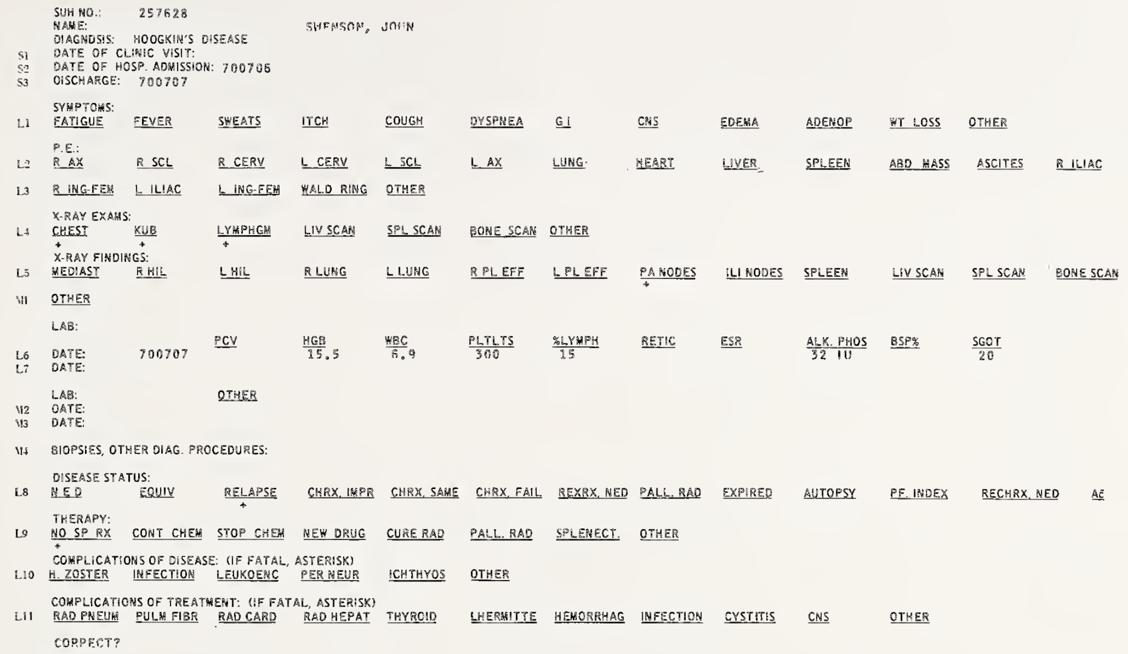
      CORRECT?
```

Text-figure 3.—Data input form for the first follow-up visit of May 6, 1968.

The patient remained apparently free of disease at other interval follow-up examinations (omitted here for brevity) until the visit of July 6, 1970 (text-fig. 4), when he was admitted to the hospital for a repeat lymphangiogram to reopacify and maintain surveillance on the para-aortic and pelvic lymph nodes, from which the earlier contrast material had gradually disappeared. The lymphangiogram revealed enlargement and abnormality of the para-aortic nodes, indicated by a plus (+) on line L5. It was therefore considered that the patient had suffered a relapse of his Hodgkin's disease, indicated by a plus (+) sign under "relapse" on line L8. The relevant laboratory findings on that admission are entered on line L6.

One week later the patient was readmitted for staging laparotomy with splenectomy (text-fig. 5). The relevant surgical procedures at the time of laparotomy are indicated on line M4, together with the significant histologic findings: the presence of nodular-sclerosing Hodgkin's disease in the spleen and splenic hilar nodes and negative biopsies of the liver, para-aortic lymph node, and bone marrow. The patient is now scored on line L8 as alive with disease ("Ac̄"), and on the following line the decision to undertake intensive radiotherapy with curative intent ("CURE RAD") is indicated by a plus (+).

Course No. 2 of radiotherapy is then entered on a treatment-file input data form as before



```
      SUH NO.:      257628
      NAME:                                SWENSON, JOHN
      DIAGNDSIS:   HOOGKIN'S DISEASE
S1    DATE OF CLINIC VISIT:
S2    DATE OF HOSP. ADMISSION: 700706
S3    OISCHARGE:    700707

      SYMPTOMS:
L1    FATIGUE    FEVER      SWEATS     ITCH       COUGH      DYSPNEA    G I        CNS        EDEMA      ADENOP     WT LOSS    OTHER

      P.E.:
L2    R AX       R SCL      R CERV     L CERV     L SCL      L AX       LUNG       HEART      LIVER      SPLEEN     ABD MASS   ASCITES    R ILIAC

L3    R ING-FEM  L ILIAC    L ING-FEM  WALD RING  OTHER

      X-RAY EXAMS:
L4    CHEST      KUB        LYMPHGM    LIV SCAN   SPL SCAN   BONE SCAN  OTHER
       +          +          +
      X-RAY FINDINGS:
L5    MEDIAST    R HIL      L HIL      R LUNG     L LUNG     R PL EFF   L PL EFF   PA NODES   ILI NODES  SPLEEN     LIV SCAN   SPL SCAN   BONE SCAN
                                                                                     +
M1    OTHER

      LAB:
                            PCV        HGB        WBC        PLTLTS     %LYMPH     RETIC      ESR        ALK. PHOS  BSP%       SGOT
L6    DATE:      700707                15.5        6.9        300        15                               32 IU                  20
L7    DATE:

      LAB:                  OTHER
M2    OATE:
M3    DATE:

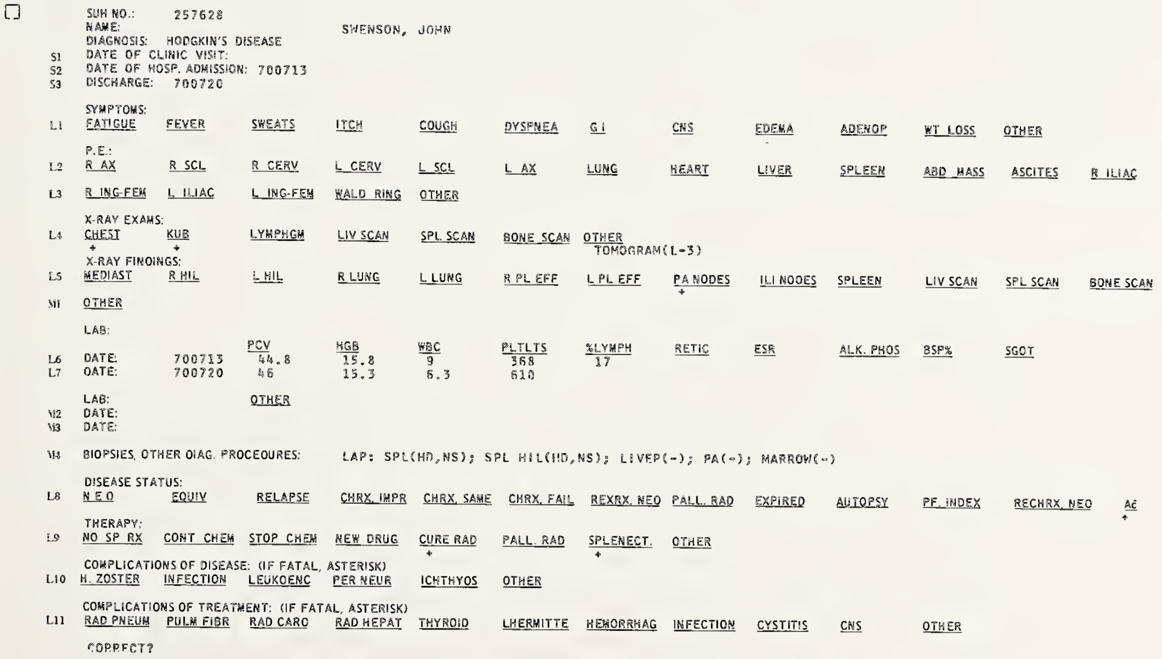
M4    BIOPSIES, OTHER DIAG. PROCEDURES:

      DISEASE STATUS:
L8    N E D      EQUIV      RELAPSE    CHRX. IMPR CHRX. SAME CHRX. FAIL REXRX. NED PALL. RAD  EXPIRED    AUTOPSY    PF. INDEX  RECHRX. NED  AE
                             +
      THERAPY:
L9    NO SP RX   CONT CHEM  STOP CHEM  NEW DRUG   CURE RAD   PALL. RAD  SPLENECT.  OTHER
       +
      COMPLICATIONS OF DISEASE:  (IF FATAL, ASTERISK)
L10   H. ZOSTER  INFECTION  LEUKOENC   PER NEUR   ICHTHYOS   OTHER

      COMPLICATIONS OF TREATMENT:  (IF FATAL, ASTERISK)
L11   RAD PNEUM  PULM FIBR  RAD CARD   RAD HEPAT  THYROID    LHERMITTE  HEMORRHAG  INFECTION  CYSTITIS   CNS        OTHER

      CORPECT?
```

TEXT-FIGURE 4.—Hospital admission for repeat lymphangiogram, which revealed positive para-aortic lymph nodes.



```
      SUH NO.:      257628
      NAME:                                SWENSON, JOHN
      DIAGNOSIS:   HODGKIN'S DISEASE
S1    DATE OF CLINIC VISIT:
S2    DATE OF HOSP. ADMISSION: 700713
S3    DISCHARGE:    700720

      SYMPTOMS:
L1    FATIGUE    FEVER      SWEATS     ITCH       COUGH      DYSPNEA    G I        CNS        EDEMA      ADENOP     WT LOSS    OTHER

      P.E.:
L2    R AX       R SCL      R CERV     L CERV     L SCL      L AX       LUNG       HEART      LIVER      SPLEEN     ABD MASS   ASCITES    R ILIAC

L3    R ING-FEM  L ILIAC    L ING-FEM  WALD RING  OTHER

      X-RAY EXAMS:
L4    CHEST      KUB        LYMPHGM    LIV SCAN   SPL SCAN   BONE SCAN  OTHER
       +          +                                                      TOMOGRAM(L-3)
      X-RAY FINOINGS:
L5    MEDIAST    R HIL      L HIL      R LUNG     L LUNG     R PL EFF   L PL EFF   PA NODES   ILI NOOES  SPLEEN     LIV SCAN   SPL SCAN   BONE SCAN
                                                                                     +
M1    OTHER

      LAB:
                            PCV        HGB        WBC        PLTLTS     %LYMPH     RETIC      ESR        ALK. PHOS  BSP%       SGOT
L6    DATE:      700713      44.8       15.8       9          368        17
L7    OATE:      700720      46         15.3       6.3        610

      LAB:                  OTHER
M2    DATE:
M3    DATE:

M4    BIOPSIES, OTHER OIAG. PROCEORES:     LAP: SPL(HD,NS); SPL HIL(HD,NS); LIVEP(-); PA(-); MARROW(-)

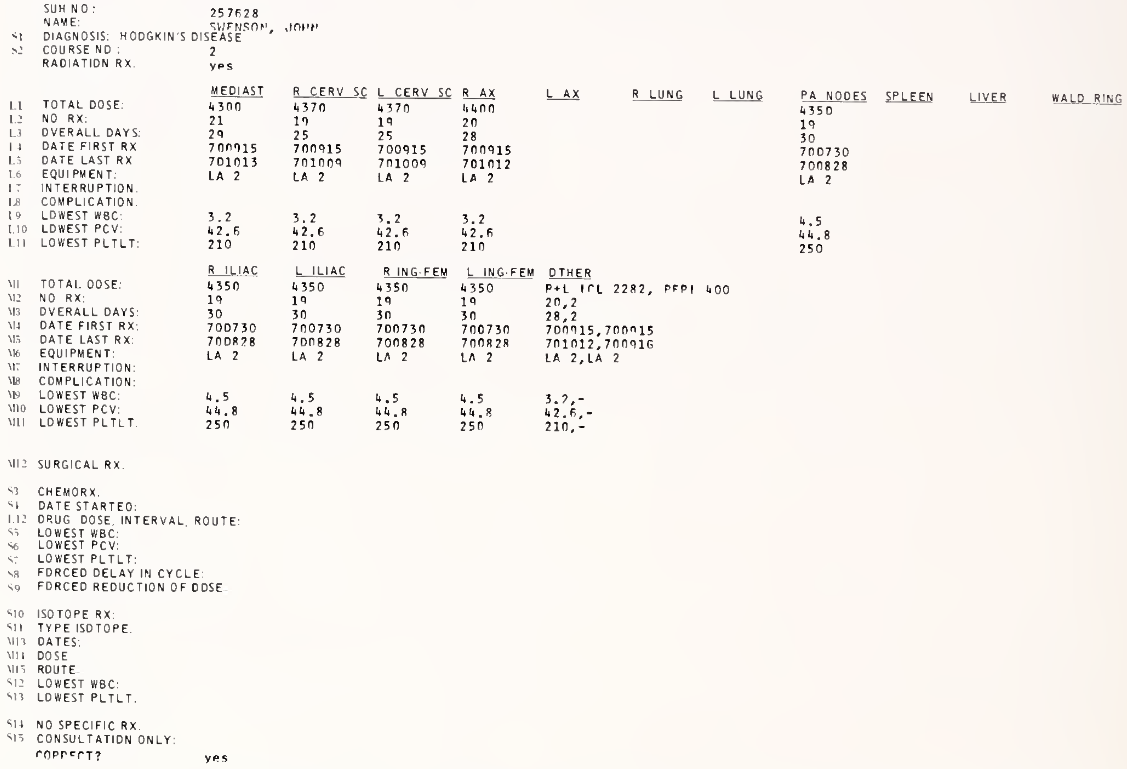
      DISEASE STATUS:
L8    N E O      EQUIV      RELAPSE    CHRX. IMPR CHRX. SAME CHRX. FAIL REXRX. NEO PALL. RAD  EXPIRED    AUTOPSY    PF. INDEX  RECHRX. NEO  AE
                                                                                                                                       +
      THERAPY:
L9    NO SP RX   CONT CHEM  STOP CHEM  NEW DRUG   CURE RAD   PALL. RAD  SPLENECT.  OTHER
                                                   +                      +
      COMPLICATIONS OF DISEASE:  (IF FATAL, ASTERISK)
L10   H. ZOSTER  INFECTION  LEUKOENC   PER NEUR   ICHTHYOS   OTHER

      COMPLICATIONS OF TREATMENT:  (IF FATAL, ASTERISK)
L11   RAD PNEUM  PULM FIBR  RAD CARO   RAD HEPAT  THYROID    LHERMITTE  HEMORRHAG  INFECTION  CYSTITIS   CNS        OTHER

      CORRECT?
```

TEXT-FIGURE 5.—Hospital admission for staging laparotomy and splenectomy, which revealed disease in the spleen and splenic hilar nodes.

(text-fig. 6). All lymph-node-bearing areas, other than the left axilla, were treated on this occasion, beginning with the infradiaphragmatic lymph nodes and concluding with the mediastinum, right axilla, and both cervical-supraclavicular regions. The nadir white blood cell count of 3,200 was reached during the latter part of this treatment regimen, as was the nadir platelet count of 210,000. There were no significant interruptions or complications of treatment, which was completed on October 13, 1970 (line L15, 701013).



```
      SUH NO:                  257628
      NAME:                    SWENSON, JOHN
S1    DIAGNOSIS: HODGKIN'S DISEASE
S2    COURSE ND:               2
      RADIATIDN RX.            yes

                               MEDIAST   R CERV SC L CERV SC R AX      L AX      R LUNG    L LUNG     PA NODES SPLEEN   LIVER     WALD RING
L1    TOTAL DOSE:              4300      4370      4370      4400                                     4350
L2    NO RX:                   21        19        19        20                                       19
L3    DVERALL DAYS:            29        25        25        28                                       30
L4    DATE FIRST RX            700915    700915    700915    700915                                   700730
L5    DATE LAST RX             701013    701009    701009    701012                                   700828
L6    EQUIPMENT:               LA 2      LA 2      LA 2      LA 2                                     LA 2
L7    INTERRUPTION.
L8    COMPLICATION.
L9    LDWEST WBC:              3.2       3.2       3.2       3.2                                      4.5
L10   LDWEST PCV:              42.6      42.6      42.6      42.6                                     44.8
L11   LOWEST PLTLT:            210       210       210       210                                      250

                               R ILIAC   L ILIAC   R ING-FEM L ING-FEM DTHER
M1    TOTAL OOSE:              4350      4350      4350      4350      P+L ICL 2282, PERI 400
M2    NO RX:                   19        19        19        19        20,2
M3    DVERALL DAYS:            30        30        30        30        28,2
M4    DATE FIRST RX:           700730    700730    700730    700730    700915,700915
M5    DATE LAST RX:            700828    700828    700828    700828    701012,70091G
M6    EQUIPMENT:               LA 2      LA 2      LA 2      LA 2      LA 2,LA 2
M7    INTERRUPTION:
M8    CDMPLICATION:
M9    LOWEST WBC:              4.5       4.5       4.5       4.5       3.2,-
M10   LOWEST PCV:              44.8      44.8      44.8      44.8      42.6,-
M11   LDWEST PLTLT.            250       250       250       250       210,-

M12   SURGICAL RX.

S3    CHEMORX.
S4    DATE STARTEO:
L12   DRUG DOSE, INTERVAL, ROUTE:
S5    LOWEST WBC:
S6    LOWEST PCV:
S7    LOWEST PLTLT:
S8    FDRCED DELAY IN CYCLE:
S9    FDRCED REDUCTION OF DDSE.

S10   ISOTOPE RX:
S11   TYPE ISDTOPE.
M13   DATES:
M14   DOSE
M15   RDUTE.
S12   LOWEST WBC:
S13   LDWEST PLTLT.

S14   NO SPECIFIC RX.
S15   CONSULTATIDN ONLY:
      CORRECT?                 yes
```

TEXT-FIGURE 6.—Data input form for the second course of radiotherapy, covering all major lymph-node chains other than the left axilla.

After a rest period of approximately 3 months, the patient was started on the first of 6 cycles of MOPP quadruple drug chemotherapy. This treatment course is 3A through 3F (text-figs. 7–12) on line S2. On each of these, the date of starting the cycle is indicated on line S4; the respective drugs, together with their doses, the days within the 14-day cycle on which they were given, and the route of administration are given on line L12; the lowest white blood cell count recorded during the cycle is on line S5; the lowest packed cell volume on line S6; and the lowest platelet count on line S7. Forced delays in initiation of a cycle or enforced reduction of dose are indicated, when necessary, on lines S8 and S9.

Returning to the follow-up file, the input data form (text-fig. 13) indicates an interval visit on November 16, 1970, after the completion of the second course of radiotherapy and before the initiation of MOPP chemotherapy, at which time the clinical status of the patient is indicated as no evidence of disease after reirradiation (line L8, "REXRX, NED"). The follow-up file resumes after completion of MOPP chemotherapy with a visit on July 26, 1971 (text-fig. 14), at which time the patient complained of a symptom complex typical of the Lhermitte syn-

```
UH NO.:                 257628
IAME:                   SWENSON, JOHN
IAGNOSIS:  HODGKIN'S DISEASE
OURSE NO.:              3A
ADIATION RX:            NO

                       MEDIAST   R CERV SC L CERV SC R AX      L AX      R LUNG   L LUNG    PA NODES  SPLEEN    LIVER     WALD RING
TOTAL DOSE:
O. RX:
VERALL DAYS:
ATE FIRST RX:
ATE LAST RX:
EQUIPMENT:
INTERRUPTION:
COMPLICATION:
LOWEST WBC:
LOWEST PCV:
LOWEST PLTLT:

                       R ILIAC    L ILIAC    R ING-FEM  L ING-FEM  OTHER
TOTAL DOSE:
NO. RX:
OVERALL DAYS:
DATE FIRST RX:
DATE LAST RX:
EQUIPMENT:
INTERRUPTION:
COMPLICATION:
LOWEST WBC:
LOWEST PCV:
LOWEST PLTLT:


SURGICAL RX:

CHEMORX:                MOPP
DATE STARTED:           710118
DRUG, DOSE, INTERVAL, ROUTE:        HN2;13;1,8;IV: VIN;2;1,8;IV: PROC;200;1-14;PO: PRED;90;1-14;PO
LOWEST WBC:             5.9
LOWEST PCV:             45.2
LOWEST PLTLT:           350
FORCED DELAY IN CYCLE:
FORCED REDUCTION OF DOSE:

ISOTOPE RX:
TYPE ISOTOPE:
DATES:
DOSE:
ROUTE:
LOWEST WBC:
LOWEST PLTLT:

NO SPECIFIC RX:
CONSULTATION ONLY:
  CORRECT?
```

Text-figure 7.—Data input form for cycle 1 of MOPP combination therapy.

```
     SUH NO.:                 257628
     NAME:                    SWENSON, JOHN
S1   DIAGNOSIS:  HODGKIN'S DISEASE
S2   COURSE NO.:              3B
     RADIATION RX:            NO

                              MEDIAST   R CERV SC L CERV SC R AX      L AX      R LUNG   L LUNG    PA NODES  SPLEEN    LIVER     WALD RING
L1   TOTAL DOSE:
L2   NO. RX:
L3   OVERALL DAYS:
L4   DATE FIRST RX:
L5   DATE LAST RX:
L6   EQUIPMENT:
L7   INTERRUPTION:
L8   COMPLICATION:
L9   LDWEST WBC:
L10  LOWEST PCV:
L11  LOWEST PLTLT:

                              R ILIAC    L ILIAC    R ING-FEM  L ING-FEM  OTHER
M1   TOTAL DOSE:
M2   NO. RX:
M3   OVERALL DAYS:
M4   DATE FIRST RX:
M5   DATE LAST RX:
M6   EQUIPMENT:
M7   INTERRUPTION:
M8   COMPLICATION:
M9   LOWEST WBC:
M10  LOWEST PCV:
M11  LOWEST PLTLT:

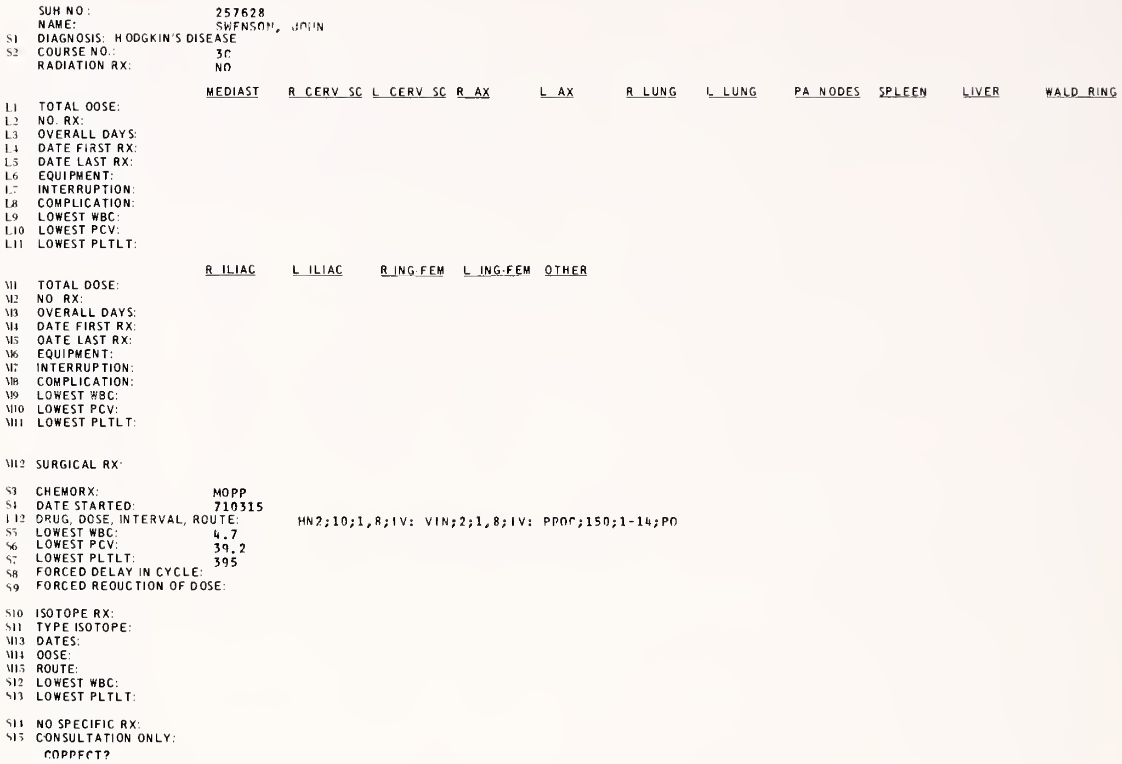

M12  SURGICAL RX:

S3   CHEMORX:                 MOPP
S4   DATE STARTED:            710216
L12  DRUG, OOSE, INTERVAL, ROUTE:        HN2;10;1,7;IV: VIN;2;1,7;IV: PROC;150;1-14;PO
S5   LOWEST WBC:              3.2
S6   LOWEST PCV:              42.9
S7   LOWEST PLTLT:            295
S8   FORCED DELAY IN CYCLE:
S9   FORCED REDUCTION OF DOSE:           +

S10  ISOTDPE RX:
S11  TYPE ISDTOPE:
M13  DATES:
M14  DOSE:
M15  ROUTE:
S12  LOWEST WBC:
S13  LOWEST PLTLT:

S14  NO SPECIFIC RX:
S15  CONSULTATION ONLY:
       CORRECT?
```

Text-figure 8.—Data input form for cycle 2 of MOPP combination therapy.



```
         SUH NO:                       257628
         NAME:                         SWFNSON, JOHN
S1       DIAGNOSIS: HODGKIN'S DISEASE
S2       COURSE NO.:                   3C
         RADIATION RX:                 NO

                                       MEDIAST   R CERV SC L CERV SC R AX      L AX       R LUNG    L LUNG     PA NODES  SPLEEN    LIVER     WALD RING
L1       TOTAL OOSE:
L2       NO. RX:
L3       OVERALL DAYS:
L4       DATE FIRST RX:
L5       DATE LAST RX:
L6       EQUIPMENT:
L7       INTERRUPTION:
L8       COMPLICATION:
L9       LOWEST WBC:
L10      LOWEST PCV:
L11      LOWEST PLTLT:

                                       R ILIAC    L ILIAC     R ING-FEM  L ING-FEM  OTHER
M1       TOTAL DOSE:
M2       NO RX:
M3       OVERALL DAYS:
M4       DATE FIRST RX:
M5       OATE LAST RX:
M6       EQUIPMENT:
M7       INTERRUPTION:
M8       COMPLICATION:
M9       LOWEST WBC:
M10      LOWEST PCV:
M11      LOWEST PLTLT:

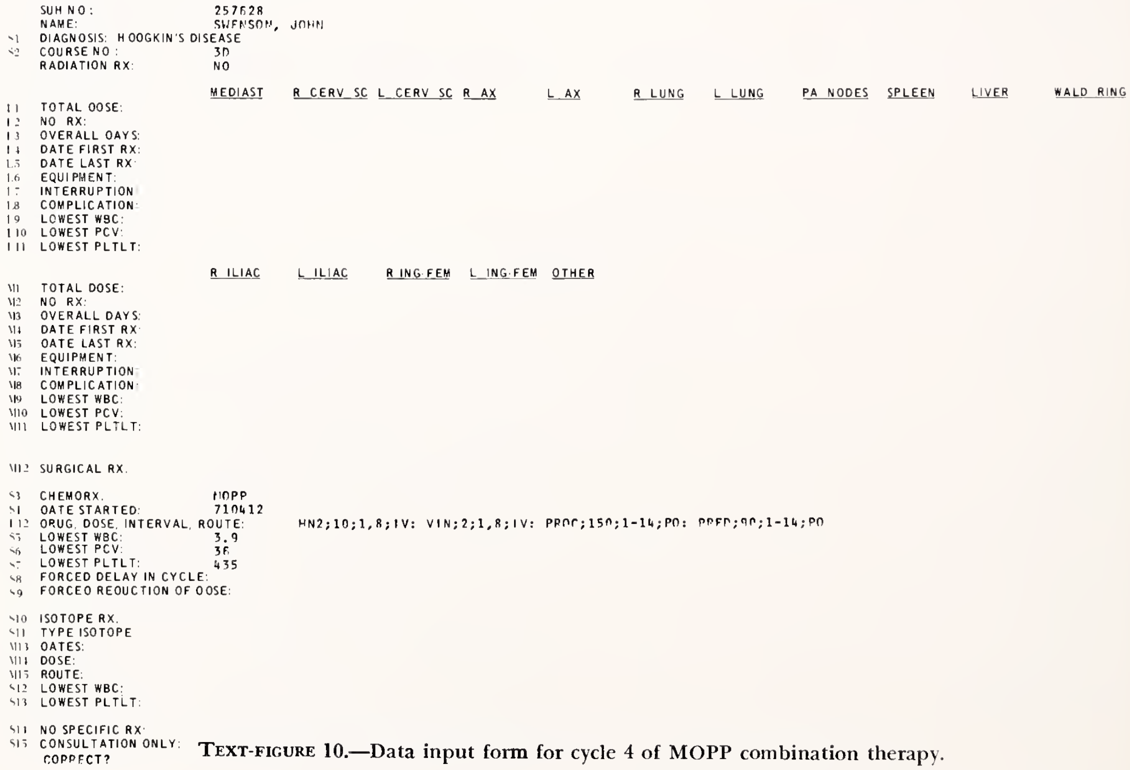

M12      SURGICAL RX:

S3       CHEMORX:                      MOPP
S4       DATE STARTED:                 710315
L12      DRUG, DOSE, INTERVAL, ROUTE:          HN2;10;1,8;IV: VIN;2;1,8;IV: PROC;150;1-14;PO
S5       LOWEST WBC:                   4.7
S6       LOWEST PCV:                   39.2
S7       LOWEST PLTLT:                 395
S8       FORCED DELAY IN CYCLE:
S9       FORCED REOUCTION OF DOSE:

S10      ISOTOPE RX:
S11      TYPE ISOTOPE:
M13      DATES:
M14      OOSE:
M15      ROUTE:
S12      LOWEST WBC:
S13      LOWEST PLTLT:

S14      NO SPECIFIC RX:
S15      CONSULTATION ONLY:
          COPPECT?
```

TEXT-FIGURE 9.—Data input form for cycle 3 of MOPP combination therapy.



```
         SUH NO:                       257628
         NAME:                         SWFNSON, JOHN
S1       DIAGNOSIS: HOODGKIN'S DISEASE
S2       COURSE NO :                   3D
         RADIATION RX:                 NO

                                       MEDIAST   R CERV SC L CERV SC R AX      L AX       R LUNG    L LUNG     PA NODES  SPLEEN    LIVER     WALD RING
L1       TOTAL OOSE:
L2       NO RX:
L3       OVERALL OAYS:
L4       DATE FIRST RX:
L5       DATE LAST RX:
L6       EQUIPMENT:
L7       INTERRUPTION:
L8       COMPLICATION:
L9       LOWEST WBC:
L10      LOWEST PCV:
L11      LOWEST PLTLT:

                                       R ILIAC    L ILIAC     R ING-FEM  L ING-FEM  OTHER
M1       TOTAL DOSE:
M2       NO RX:
M3       OVERALL DAYS:
M4       DATE FIRST RX:
M5       OATE LAST RX:
M6       EQUIPMENT:
M7       INTERRUPTION:
M8       COMPLICATION:
M9       LOWEST WBC:
M10      LOWEST PCV:
M11      LOWEST PLTLT:

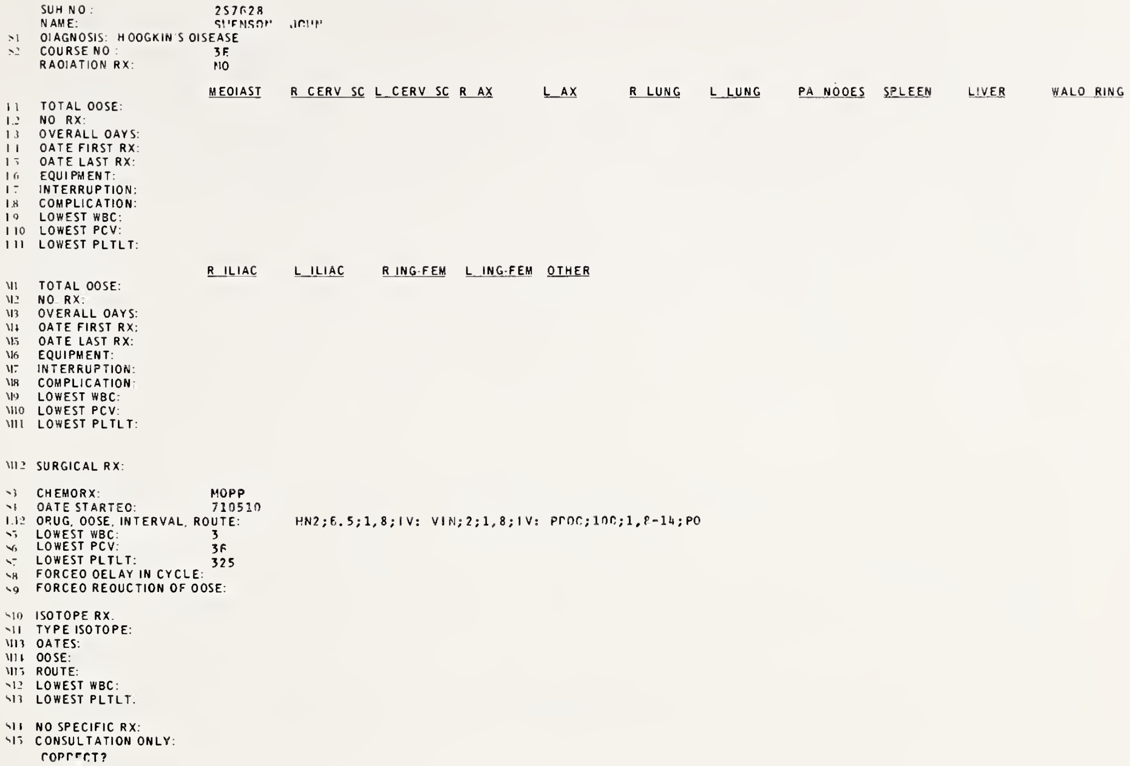

M12      SURGICAL RX.

S3       CHEMORX.                      MOPP
S4       OATE STARTED:                 710412
L12      ORUG, DOSE, INTERVAL, ROUTE:          HN2;10;1,8;IV: VIN;2;1,8;IV: PROC;150;1-14;PO: PRED;90;1-14;PO
S5       LOWEST WBC:                   3.9
S6       LOWEST PCV:                   36
S7       LOWEST PLTLT:                 435
S8       FORCED DELAY IN CYCLE:
S9       FORCEO REOUCTION OF OOSE:

S10      ISOTOPE RX.
S11      TYPE ISOTOPE
M13      OATES:
M14      DOSE:
M15      ROUTE:
S12      LOWEST WBC:
S13      LOWEST PLTLT:

S14      NO SPECIFIC RX:
S15      CONSULTATION ONLY:
          COPPECT?
```

TEXT-FIGURE 10.—Data input form for cycle 4 of MOPP combination therapy.



```
      SUH NO:                257628
      NAME:                  SVENSON, JOHN
S1    OIAGNOSIS:  HOOGKIN'S OISEASE
S2    COURSE NO:             3E
      RAOIATION RX:          NO

                             MEOIAST   R CERV SC L CERV SC R AX     L AX     R LUNG   L LUNG    PA NOOES  SPLEEN   LIVER    WALO RING
L1    TOTAL OOSE:
L2    NO RX:
L3    OVERALL OAYS:
L4    OATE FIRST RX:
L5    OATE LAST RX:
L6    EQUIPMENT:
L7    INTERRUPTION:
L8    COMPLICATION:
L9    LOWEST WBC:
L10   LOWEST PCV:
L11   LOWEST PLTLT:

                             R ILIAC   L ILIAC    R ING-FEM  L ING-FEM  OTHER
M1    TOTAL OOSE:
M2    NO RX:
M3    OVERALL OAYS:
M4    OATE FIRST RX:
M5    OATE LAST RX:
M6    EQUIPMENT:
M7    INTERRUPTION:
M8    COMPLICATION:
M9    LOWEST WBC:
M10   LOWEST PCV:
M11   LOWEST PLTLT:

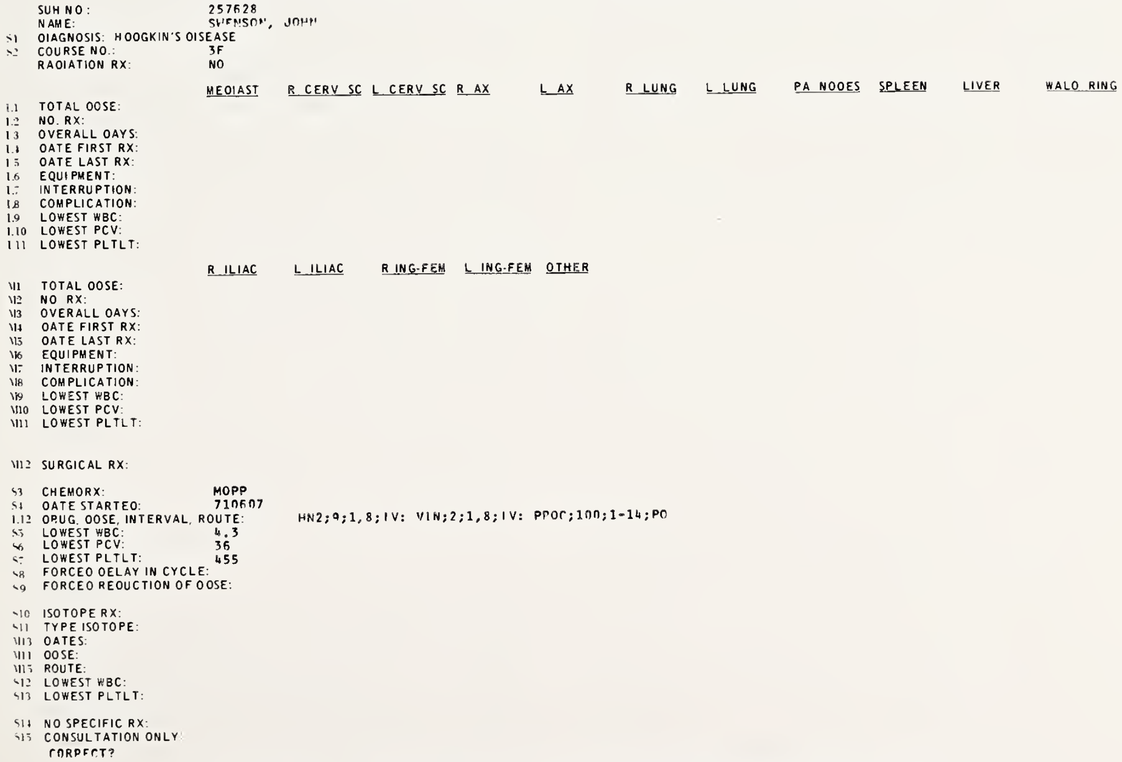

M12   SURGICAL RX:

S3    CHEMORX:               MOPP
S4    OATE STARTEO:          710510
L12   ORUG, OOSE, INTERVAL, ROUTE:       HN2;6.5;1,8;IV: VIN;2;1,8;IV: PROC;100;1,8-14;PO
S5    LOWEST WBC:            3
S6    LOWEST PCV:            36
S7    LOWEST PLTLT:          325
S8    FORCEO OELAY IN CYCLE:
S9    FORCEO REOUCTION OF OOSE:

S10   ISOTOPE RX.
S11   TYPE ISOTOPE:
M13   OATES:
M14   OOSE:
M15   ROUTE:
S12   LOWEST WBC:
S13   LOWEST PLTLT.

S14   NO SPECIFIC RX:
S15   CONSULTATION ONLY:
       CORRECT?
```

TEXT-FIGURE 11.—Data input form for cycle 5 of MOPP combination therapy.



```
      SUH NO:                257628
      NAME:                  SVENSON, JOHN
S1    OIAGNOSIS:  HOOGKIN'S OISEASE
S2    COURSE NO.:            3F
      RAOIATION RX:          NO
                             MEOIAST   R CERV SC L CERV SC R AX     L AX     R LUNG   L LUNG    PA NOOES  SPLEEN   LIVER    WALO RING
L1    TOTAL OOSE:
L2    NO. RX:
L3    OVERALL OAYS:
L4    OATE FIRST RX:
L5    OATE LAST RX:
L6    EQUIPMENT:
L7    INTERRUPTION:
L8    COMPLICATION:
L9    LOWEST WBC:
L10   LOWEST PCV:
L11   LOWEST PLTLT:
                             R ILIAC   L ILIAC    R ING-FEM  L ING-FEM  OTHER
M1    TOTAL OOSE:
M2    NO RX:
M3    OVERALL OAYS:
M4    OATE FIRST RX:
M5    OATE LAST RX:
M6    EQUIPMENT:
M7    INTERRUPTION:
M8    COMPLICATION:
M9    LOWEST WBC:
M10   LOWEST PCV:
M11   LOWEST PLTLT:

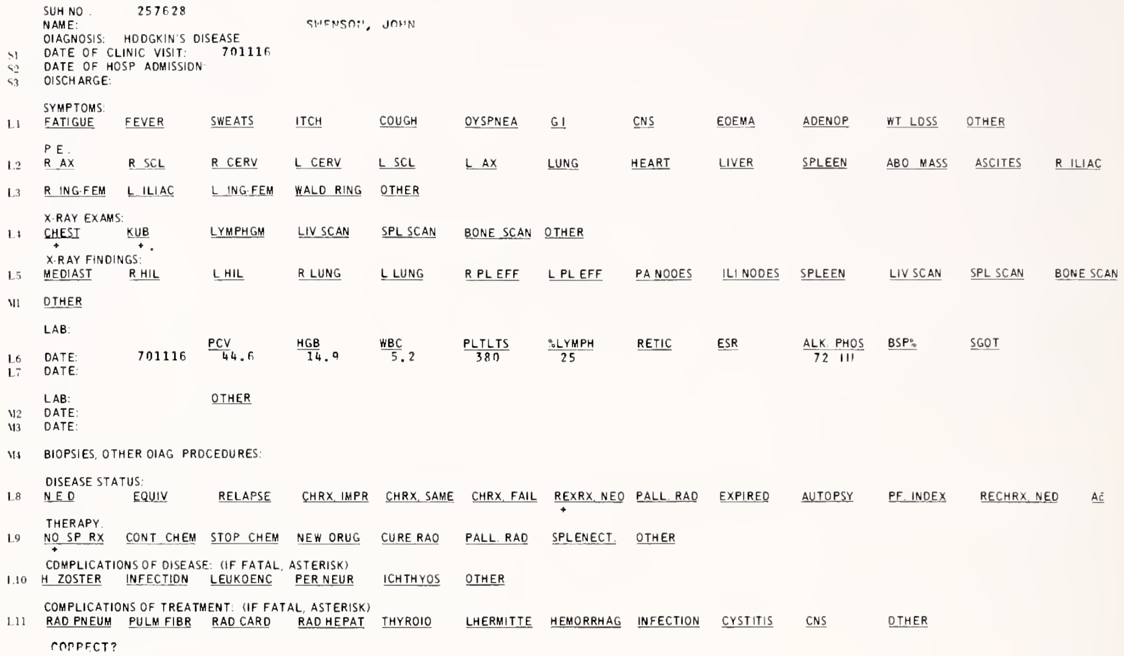

M12   SURGICAL RX:

S3    CHEMORX:               MOPP
S4    OATE STARTEO:          710607
L12   ORUG, OOSE, INTERVAL, ROUTE:       HN2;9;1,8;IV: VIN;2;1,8;IV: PROC;100;1-14;PO
S5    LOWEST WBC:            4.3
S6    LOWEST PCV:            36
S7    LOWEST PLTLT:          455
S8    FORCEO OELAY IN CYCLE:
S9    FORCEO REOUCTION OF OOSE:

S10   ISOTOPE RX:
S11   TYPE ISOTOPE:
M13   OATES:
M14   OOSE:
M15   ROUTE:
S12   LOWEST WBC:
S13   LOWEST PLTLT:

S14   NO SPECIFIC RX:
S15   CONSULTATION ONLY:
       CORRECT?
```

TEXT-FIGURE 12.—Data input form for cycle 6 of MOPP combination therapy.



```
     SUH NO .      257628
     NAME:                                   SVFNSON, JOHN
     OIAGNOSIS:  HDDGKIN'S DISEASE
S1   DATE OF CLINIC VISIT:    701116
S2   DATE OF HOSP ADMISSIDN
S3   OISCHARGE:

     SYMPTOMS:
L1   FATIGUE   FEVER     SWEATS    ITCH      COUGH     OYSPNEA   G I       CNS       EOEMA     ADENOP    WT LDSS   OTHER

     P E .
L2   R AX      R SCL     R CERV    L CERV    L SCL     L AX      LUNG      HEART     LIVER     SPLEEN    ABO MASS  ASCITES   R ILIAC

L3   R ING-FEM L ILIAC   L ING-FEM WALD RING OTHER

     X-RAY EXAMS:
L4   CHEST     KUB       LYMPHGM   LIV SCAN  SPL SCAN  BONE SCAN OTHER
      +          + .
     X-RAY FINDINGS:
L5   MEDIAST   R HIL     L HIL     R LUNG    L LUNG    R PL EFF  L PL EFF  PA NOOES  ILI NODES SPLEEN    LIV SCAN  SPL SCAN  BONE SCAN

M1   DTHER

     LAB:
                         PCV       HGB       WBC       PLTLTS    %LYMPH    RETIC     ESR       ALK PHOS  BSP%      SGOT
L6   DATE:     701116     44.6      14.9      5.2       380       25                            72 III
L7   DATE:

     LAB:                OTHER
M2   DATE:
M3   DATE:

M4   BIOPSIES, OTHER OIAG PRDCEDURES:

     DISEASE STATUS:
L8   N E D     EQUIV     RELAPSE   CHRX IMPR CHRX SAME CHRX FAIL REXRX NEO PALL RAD  EXPIRED   AUTOPSY   PF INDEX  RECHRX NED  Ac
                                                        +
     THERAPY.
L9   NO SP RX  CONT CHEM STOP CHEM NEW ORUG  CURE RAO  PALL RAD  SPLENECT. OTHER
      +
     CDMPLICATIONS OF DISEASE: (IF FATAL, ASTERISK)
L10  H ZOSTER  INFECTIDN LEUKOENC  PER NEUR  ICHTHYOS  OTHER

     COMPLICATIONS OF TREATMENT: (IF FATAL, ASTERISK)
L11  RAD PNEUM PULM FIBR RAD CARD  RAD HEPAT THYROIO   LHERMITTE HEMORRHAG INFECTION CYSTITIS  CNS       DTHER

      COPPECT?
```

TEXT-FIGURE 13.—Interval follow-up visit of November 16, 1970.

drome, indicated by a plus (+) at the appropriate place on line L11; but the patient had a Karnofsky performance index of 100 and was considered to be without evidence of disease after retreatment with chemotherapy ("RECHRX, NED"). The final clinical visit to date occurred November 29, 1971, at which time the patient remained without evidence of disease and was asymptomatic (text-fig. 15).

RETRIEVAL

The retrieval program was designed to provide the following types of information: 1) all or part of a patient's computer record; 2) based on the parent file, a new computer file, of selected records, can be created, saved, and interrogated independently; and 3) actuarial survival statistics. In addition, some accessory programs for retrieval of certain specific types of information are in use and others are being developed as the need arises.

The salient features of this retrieval program can be demonstrated by a review of the disease-free survival statistics of a subset of patients who were chosen on the basis of a number of cross-correlations. The example chosen is the group of patients found to have Stage I-A or II-A disease who received involved-field irradiation only. These patients are represented by the protocols L1A and H1A.

To obtain the survival statistics, it is necessary to express the parameters, stage and symptoms, in a codified statement ("Condition list," table 1). In this instance, the first 2 variables, A1 and A2, refer in code to entries on line L12 in text-figure 1 to specify Stage I and II disease, treated by local radiotherapy only, as represented by the protocols L1A and H1A. The third variable, A3, indicates our interest in "A" patients with no constitutional symptoms. The powerful part of this program is that, having defined these variables, we can combine them in any way we wish by using a conditional expression. In this example, the correct expression is shown as "[COND EXPR] = ?(A1 or A2) & A3." This, in effect, states Stage I or II disease and "A" cases without symptoms. Different conditional expressions using the same variables can be used to retrieve yet another subset of patients. Up to 60 variables representing patient parameters can be defined in any one interrogation, and the possible number of logical combinations of these variables is

```
     SUH NO.:    257628
     NAME:                               SWENSON, JOHN
     DIAGNOSIS:  HODGKIN'S DISEASE
S1   DATE OF CLINIC VISIT:   710726
S2   DATE OF HDSP ADMISSION:
S3   DISCHARGE:

     SYMPTOMS.
L1   FATIGUE     FEVER       SWEATS      ITCH        COUGH       DYSPNEA     G I         CNS         EDEMA       ADENOP      WT LDSS     OTHER
                                                                                          +
     P E.
L2   R AX        R SCL       R CERV      L CERV      L SCL       L AX        LUNG        HEART       LIVER       SPLEEN      ABD MASS    ASCITES     R ILIAC

L3   R ING-FEM   L ILIAC     L ING-FEM   WALD RING   OTHER

     X-RAY EXAMS:
L4   CHEST       KUB         LYMPHGM     LIV SCAN    SPL SCAN    BONE SCAN   DTHER
      +           +
     X-RAY FINDINGS:
L5   MEDIAST     R HIL       L HIL       R LUNG      L LUNG      R PL EFF    L PL EFF    PA NODES    ILI NODES   SPLEEN      LIV SCAN    SPL SCAN    BDNE SCAN

M1   OTHER

     LAB:
                             PCV         HGB         WBC         PLTLTS      %LYMPH      RETIC       ESR         ALK. PHOS   BSP%        SGOT
L6   DATE:       710726        43.8       14.6         5.4         400         30                      8            59 IU
L7   DATE:

     LAB:                    DTHER
M2   DATE:
M3   DATE:

M4   BIOPSIES, OTHER DIAG PROCEDURES:

     DISEASE STATUS:
L8   N E D       EQUIV       RELAPSE     CHRX. IMPR  CHRX. SAME  CHRX. FAIL  REXRX. NED  PALL RAD    EXPIRED     AUTOPSY     PF. INDEX   RECHRX. NED AC
                                                                                                                  100
     THERAPY:
L9   NO SP RX    CONT CHEM   STOP CHEM   NEW DRUG    CURE RAD    PALL. RAD   SPLENECT.   OTHER
      +
     COMPLICATIONS OF DISEASE: (IF FATAL, ASTERISK)
L10  H ZDSTER    INFECTION   LEUKOENC    PER NEUR    ICHTHYOS    OTHER

     COMPLICATIONS OF TREATMENT. (IF FATAL, ASTERISK)
L11  RAD PNEUM   PULM FIBR   RAD CARD    RAD HEPAT   THYROID     LHERMITTE   HEMORRHAG   INFECTION   CYSTITIS    CNS         OTHER
                                                                  +
     COPRECT?
```

Text-figure 14.—Post-MOPP and radiotherapy follow-up visit of July 26, 1971. Lhermitte syndrome present.

```
     SUH ND :    257628
     NAME:                               SWENSON, JOHN
     DIAGNOSIS:  HODGKIN'S DISEASE
S1   DATE OF CLINIC VISIT:   711129
S2   DATE OF HDSP ADMISSIDN
S3   DISCHARGE:

     SYMPTDMS:
L1   FATIGUE     FEVER       SWEATS      ITCH        COUGH       DYSPNEA     G I         CNS         EDEMA       ADENDP      WT LOSS     OTHER

     P.E.:
L2   R AX        R SCL       R CERV      L CERV      L SCL       L AX        LUNG        HEART       LIVER       SPLEEN      ABD MASS    ASCITES     R ILIAC

L3   R ING-FEM   L ILIAC     L ING-FEM   WALD RING   DTHER

     X-RAY EXAMS:
L4   CHEST       KUB         LYMPHGM     LIV SCAN    SPL SCAN    BONE SCAN   OTHER
      +           +
     X-RAY FINDINGS:
L5   MEDIAST     R HIL       L HIL       R LUNG      L LUNG      R PL EFF    L PL EFF    PA NODES    ILI NODES   SPLEEN      LIV SCAN    SPL SCAN    BONE SCAN

M1   OTHER

     LAB:
                             PCV         HGB         WBC         PLTLTS      %LYMPH      RETIC       ESR         ALK. PHOS   BSP%        SGOT
L6   DATE:       711129        45.8       14.9         7.2         355         29                      6            66 IU
L7   DATE.

     LAB:                    DTHER
M2   DATE:
M3   DATE:

M4   BIOPSIES, OTHER DIAG. PRDCEDURES:

     DISEASE STATUS:
L8   N E D       EQUIV       RELAPSE     CHRX. IMPR  CHRX. SAME  CHRX. FAIL  REXRX. NED  PALL RAD    EXPIRED     AUTOPSY     PF. INDEX   RECHRX. NED AC
                                                                                                                              +
     THERAPY:
L9   NO SP RX    CDNT CHEM   STOP CHEM   NEW DRUG    CURE RAD    PALL. RAD   SPLENECT    DTHER
      +
     COMPLICATIONS DF DISEASE. (IF FATAL, ASTERISK)
L10  H ZOSTER    INFECTION   LEUKOENC    PER NEUR    ICHTHYOS    OTHER

     COMPLICATIONS OF TREATMENT: (IF FATAL ASTERISK)
L11  RAD PNEUM   PULM FIBR   RAD CARD    RAD HEPAT   THYROID     LHERMITTE   HEMORRHAG   INFECTION   CYSTITIS    CNS         DTHER

     COPPECT?
```

Text-figure 15.—Final follow-up visit to date.

impressive. The condition list further specifies that actuarial analysis of survival, "[ACTUARIAL] = ?YES," is desired, and that the analysis is to be limited to disease-free survival of patients conforming to the parameters cited who were admitted during the interval between January 1, 1960, and January 1, 1972, with a follow-up cutoff date of March 1, 1972.

Table 2 is a sample computer printout of selected pretreatment data listing codified data of 5 patients whose computer records satisfied the criteria detailed in the conditional expression stated in table 1. For example, the second patient listed is Dwight Johnson, hospital record number 195281, a male (M) born May 12, 1932 (320512), first seen at Stanford University Hospital on December 23, 1965, with line L12 entries indicating a CS of 3(S)A and a PS of 2AM–H–S–, who was randomly assigned to the L1A protocol. Note that no entries appear in items 3: through 9: of line L12 for this patient.

TABLE 1.—*Condition list*

```
[ID] = [LINE], [LINE ID], [ELEMENT], [VALUE], [FLAG]
A1 = ?140, L12, 2, L1A, 1
A2 = ?140, L12, 2, H1A, 1
A3 = ?140, L12, 1, A, 1
[COND EXPR] = ?(A1 or A2) & A3
[ACTUARIAL] = ?YES
TOTAL SURVIVAL (1), DISEASE-FREE SURVIVAL (2), OR BOTH (3) = ?2
= '[INTERVAL]';
BEGINNING DATE BETWEEN = ?600101
AND = ?720301
LATEST DATE UP TO + INCLUDING = ?720101
```

TABLE 2.—*Patient data*

```
|257628|SWENSON, JOHN|680209|M|350924|
|L12|1:1A|2:L1A|3:|4:|5:|6:|7:|8:|9:|10:1AM–|

|195281|JOHNSON, DWIGHT|651223|M|320512|
|L12|1:3(S)A|2:L1A|3:|4:|5:|6:|7:|8:|9:|10:2AM–H–S–|

|121734|JENSEN, RICHARD|640123|M|400325|
|L12|1:2A|2:L1A|3:|4:|5:|6:|7:|8:|9:|10:2AM–H–|

|239620|RICHARDS, GORDON G.|670710|M|430526|
|L12|1:1A|2:L1A|3:|4:|5:|6:|7:|8:|9:|10:1AM–|

|310533|ROGERS, DANIEL R.|691110|M|560215|
|L12|1:2A|2:L1A|3:|4:|5:|6:|7:|8:|9:|10:2AM–H–S–N+|
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Table 3 is a sample computer printout of the disease-free actuarial survival of all patients whose computer records satisfied the specified criteria. There were 60 such patients out of a total of 218 in the file. The actuarial survival computation is based on the method of Berkson and Gage (*2*), as restated by the American Joint Committee for Cancer Staging and End Results Reporting (*3*). During the 5th year (x) of observation, for example, there were 22 patients disease-free at the beginning of the interval [a1(x)], 1 relapse [r(x)], none lost to follow-up [u(x)], and 6 patients withdrawn disease-free during the interval [w(x)]. The mean number of patients at risk of developing a relapse is the number at the start of the year (22) minus half the number withdrawn (6/2 = 3), or 19, of which 1 relapsed for a relapse rate during the 5th year of 5.3%. Note that the relapse rate falls from 23.3% in the 1st year to 18.0% in the 2d, 9.0% in the 3d, 3.8% in the 4th, and 5.3% in the 5th.

The cumulative proportion of relapse-free patients in a given year [P(x)] is the product of P(x) for the previous year and p(x), the proportion of patients remaining relapse-free during the interval year. The disease-free survival rate was 76.7% at the end of the 1st year and fell gradually thereafter to 52.2% at 5 years in this

TABLE 3.—*Disease-free survival statistics*

Total No. of matches: 60 of 218 patients

x	al(x)	r(x)	u(x)	w(x)	lp(x)	q(x)	p(x)	P(x)
1	60	14	0	0	60	0.233	0.767	0.767
2	46	8	0	3	44	0.180	0.820	0.629
3	35	3	0	3	33	0.090	0.910	0.573
4	29	1	1	5	26	0.038	0.962	0.551
5	22	1	0	6	19	0.053	0.947	0.522
6	15	0	1	7	11	0.0	1.000	0.522
7	7	1	0	2	6	0.167	0.833	0.435

x	Year of observation.
al(x)	Number of patients disease-free at beginning of interval.
r(x)	Relapses during interval.
u(x)	Lost to follow-up during interval.
w(x)	Withdrawn disease-free during interval.
lp(x)	Effective number exposed to risk of relapse during interval.
q(x)	Proportion of patients having relapses during interval.
p(x)	Proportion of patients disease-free during interval.
P(x)	Cumulative proportion of patients not having relapses from date first seen through end of interval.

hypothetical series of Stage I-A and II-A patients who received limited-field irradiation.

Because of the flexibility of this system, there are a number of areas, in addition to survival data, where it can provide useful information. Additional examples include: 1) the fraction of abnormal lymphangiograms which have not been verified by laparotomy; 2) the incidence of radiation pericarditis, and its correlation with the recorded radiation dosage to the pericardium; and 3) the initial distribution of sites of involvement versus the sites of appearance of new manifestations, for analyses of the mode of spread of the disease.

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Statistical Procedures for Evaluating Survival in Hodgkin's Disease [1]

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SUMMARY—Two statistical methods for handling multivariate data are illustrated using 270 Hodgkin's disease patients seen at the Clinical Center of the National Institutes of Health. The first procedure, based on a *relative odds* technique, provides for an evaluation of differences in survival for patients classified according to a specific factor while taking into account differences in their distribution with respect to 1 or 2 other prognostic factors. The second procedure is a multivariate method, based on the assumption of an exponential distribution, which allows for joint evaluation of all factors thought to be associated with survival. The adjustment procedure is illustrated by contrasting survival for males with that for females while adjusting for differences in distribution with respect to histologic type and age. Female survival is superior to that of males even after taking these 2 factors into account. The multivariate method based on assumption of an exponential model is used to estimate the relationship to median survival time of histologic type, sex, symptoms, and stage of disease. This type of analysis indicates a better prognosis for females and for patients with the nodular sclerosis (NS) type of Hodgkin's disease. Estimated median survival for NS patients ranges from a high of 13.5 years for asymptomatic females in an early Stage (I or II) to a low of 3.4 years for males presenting with constitutional symptoms and more advanced disease (Stage III or IV).—Natl Cancer Inst Monogr 36: 555–559, 1973.

IN AN EVALUATION of the survival outlook for patients with Hodgkin's disease, it is important to consider simultaneously, if possible, all factors of prognostic importance. Such factors as sex and age of the patient as well as the presence of constitutional symptoms, histologic subtype, and extent of involvement of the disease as individual factors, have been observed to be related to the probability of survival.

The purpose of this report is to present for persons concerned with research on Hodgkin's disease examples of the application of two recently developed statistical procedures designed for taking into account several prognostic factors simultaneously in examining survival results.

ADJUSTMENT OF SURVIVAL CURVES

In examining the superior survival for females relative to that for males [4] (text-fig. 1), one must consider whether this difference in survival patterns could be explained on the basis of a more favorable distribution in the females with respect to factors known to be related to prognosis. For example, Berard and his coworkers (*1*) found that the nodular-sclerosis (NS) type of Hodgkin's disease occurred most often in women 15–34 years of age. Since NS is—along with lymphocytic predominance (LP)—a relatively favorable histologic type of Hodgkin's disease and younger patients have better survival chances than older ones, it could be that the observed differences in survival between males and females might be explained if these factors were taken into account.

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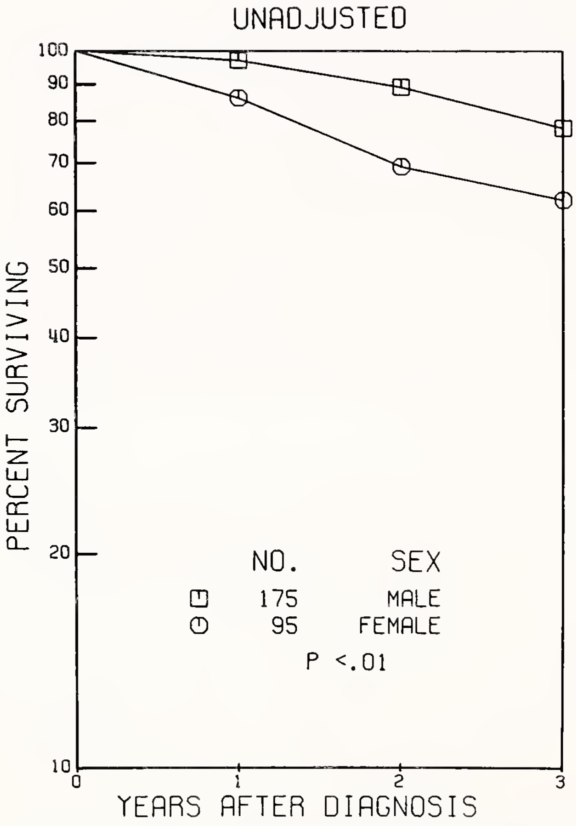
[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] We acknowledge the respective contributions of the people involved in assembling the basic data used in this paper for illustrative material: Drs. Costan W. Berard, A. Robert Kagan, Guy R. Newell, and Louis B. Thomas.

[3] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

[4] The data on Hodgkin's disease used in illustrating the statistical procedures were selected from the series of patients seen at the Clinical Center of the National Institutes of Health (NIH) from July 1953 through July 1968. From the initial 345 Hodgkin's disease patients previously discussed by Berard et al. (*1*) and Axtell et al. (*2*), we excluded 75 patients for such reasons as inadequate clinical information, diagnosis >1 year before admission to the Clinical Center, and not histologically classifiable, leaving 270 for analysis.



Text-figure 1.—Survival for male versus female Hodgkin's disease patients. Source: NIH Clinical Center series 1953–68.

Hankey and Myers (*3*) developed a method for evaluating differences in survival between two groups of patients while adjusting for one or more other factors. In describing this procedure, it is convenient to refer to the hypothetical example (table 1) of 200 patients classified with respect to a factor which divides them into group A (100 patients) and group B (100 patients). At the end of the interval of observation, the overall survival percentages are 43 and 68%, respectively, for groups A and B. It is important to note that there are no survival differences between these two groups for each specific stage, although survival varies over stage from a high of 80% in Stage I to a low of 20% in Stage IV. However, for group A only 15% of patients are in Stages I or II as compared to 65% of patients in group B.

The Hankey-Myers procedure is based on the *odds* of survival, which is the ratio of the proportion surviving to the proportion dying. This would be $p_1/(1-p_1)$ for group A and $p_2/(1-p_2)$ for group B, where p_1 and p_2 are the respective survival proportions. The *relative odds* for survival is the ratio of the odds of survival for group A to the odds of survival for group B which is:

$$R = \frac{p_1/(1-p_1)}{p_2/(1-p_2)} \text{ or } \frac{p_1\,(1-p_2)}{p_2\,(1-p_1)}$$

The procedure requires classification of patients within each stage as follows:

	Alive	Dead	Total
Group A	A_i	B_i	$A_i + B_i$
Group B	C_i	D_i	$C_i + D_i$
Total	$A_i + C_i$	$B_i + D_i$	T_i

Where *i* is the index for stage; i.e., there would be one such table for each stage. The *summary relative odds* for survival of group A to group B over the 4 stages would be:

$$\widetilde{R} = \frac{\Sigma_i(A_i D_i/T_i)}{\Sigma_i(B_i C_i/T_i)} \cdot \qquad [1]$$

An adjusted survival rate, p_2, for group B can be obtained by substituting the value of $\widetilde{R}$ calculated in equation 1 and the observed sur-

TABLE 1.—*Hypothetical example to illustrate the adjustment of survival rates*

Stage	Number of patients entering interval		Number alive at end of interval		Probability of surviving interval	
	Group A	Group B	Group A	Group B	Group A	Group B
I	10	15	8	12	0.80	0.80
II	5	50	4	40	.80	.80
III	45	30	23	15	.51	.50
IV	40	5	8	1	.20	.20
Total	100	100	43	68	.43	.68*

*$p<0.05$.

vival rate p_1 for group A in the following equation and solving for $\tilde{p}_2$:

$$\tilde{R} = \frac{p_1(1-\tilde{p}_2)}{\tilde{p}_2(1-p_1)}\,. \qquad [2]$$

For the example given in table 1, the calculations are as follows:

$$\tilde{R} = \frac{8 \cdot 3/25 + 4 \cdot 10/55 + 23 \cdot 15/75 + 8 \cdot 4/45}{12 \cdot 2/25 + 40 \cdot 1/55 + 15 \cdot 22/75 + 1 \cdot 32/45}$$

$$= \frac{6.998}{6.798} = 1.029.$$

From table 1, we find that the survival proportion for group A is

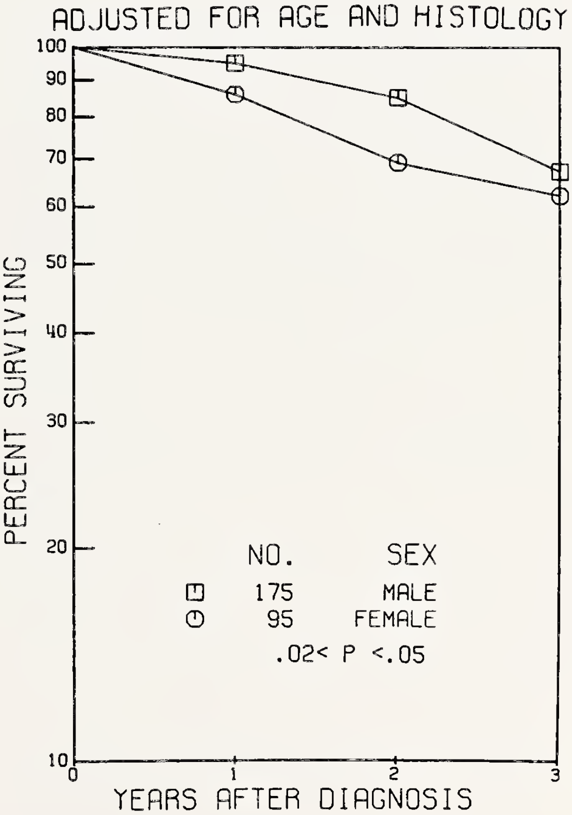
$$p_1 = 0.43.$$

Substituting these values in equation [2] we have

$$1.029 = \frac{.43(1-\tilde{p}_2)}{\tilde{p}_2(1-.43)}$$

which results in $p_2=0.42$ for group B. Thus we would conclude that the difference in survival between groups A and B could be explained on the basis of differences in distribution with respect to stage.

In a real survival comparison, one would like to adjust results not only for one interval of time but also for the total survival pattern. This is accomplished by applying the above technique to the data for each interval of follow-up. The complete, adjusted survival curve would then be obtained by cumulative multiplication of the



TEXT-FIGURE 2.—Survival for male versus female Hodgkin's disease patients with adjustment for age and histologic subtype. Source: NIH Clinical Center series, 1953–68.

adjusted survival probabilities for successive intervals.

This method is used in comparing survival for male versus female Hodgkin's disease patients while adjusting for histologic subtype (nodular sclerosis, lymphocytic predominance, mixed cellularity, and lymphocytic depletion) and age (0–14, 15–34, 35–49, 50+ yrs). The result of the adjustment is shown in text-figure 2. Taking these two factors into account did not fully explain the superior survival results observed for females.

MULTIPLE FACTOR ANALYSIS

The method presented in the previous section is useful in assessing the prognostic power of single factors while in each case adjusting for other variables, but it does not provide a means of estimating the effect on survival expectation of a combination of factors considered jointly. For this aspect of evaluating the factors, a multiple regression analysis is presented based on the exponential distribution. The probability of surviving a length of time, *t*, is

$$p = e^{-t/E}$$

where the parameter E is a linear combination of patient characteristics, i.e.,

$$E = B_0 + B_1X_1 + \dots + B_kX_k \tag{3}$$

where $X_1, X_2 \dots X_k$ are patient or disease characteristics and $B_0, B_1, \dots B_k$ are parameters to be estimated from the data.[5] For the Hodgkin's disease data, we are using the following notation

$$X_1 = \begin{cases} +1 \text{ if patient is in Stage I/II} \\ -1 \text{ if patient is in Stage III/IV} \end{cases}$$

$$X_2 = \begin{cases} +1 \text{ if symptoms were absent} \\ -1 \text{ if symptoms were present} \end{cases}$$

$$X_3 = \begin{cases} +1 \text{ if female} \\ -1 \text{ if male} \end{cases}$$

For this model, results can easily be expressed in a form so that median survival time can be estimated directly.

To illustrate this type of analysis consider the NIH Clinical Center Hodgkin's disease data for patients <50 years old who were histologically classified as 1) NS type or 2) as MC type. For *NS* patients, the procedure resulted in the following equation for estimating median survival years as a function of sex, symptoms, and stage:

$$\text{Estimated median years of survival} = 8.47 + \begin{array}{c} \textit{Sex} \\ \begin{array}{c} \text{F} \\ \text{M} \end{array} \begin{bmatrix} 2.31 \\ -2.31 \end{bmatrix} \end{array} + \begin{array}{c} \textit{Symptoms} \\ \begin{array}{c} \text{Absent} \\ \text{Present} \end{array} \begin{bmatrix} +2.15 \\ -2.15 \end{bmatrix} \end{array} + \begin{array}{c} \textit{Stage} \\ \begin{array}{c} \text{I/II} \\ \text{III/IV} \end{array} \begin{bmatrix} +0.58 \\ -0.58 \end{bmatrix} \end{array} \tag{4}$$

As an example, a female patient <50 years old, classified as having NS in clinic Stage II and presenting symptoms, has a fifty-fifty chance of surviving 9.2 years (8.47 + 2.31 — 2.15 + 0.58 = 9.21).

The median survival relationship for *MC* patients is:

$$\text{Estimated median years of survival} = 4.08 + \begin{array}{c} \textit{Sex} \\ \begin{array}{c} \text{F} \\ \text{M} \end{array} \begin{bmatrix} +0.42 \\ -0.42 \end{bmatrix} \end{array} + \begin{array}{c} \textit{Symptoms} \\ \begin{array}{c} \text{Absent} \\ \text{Present} \end{array} \begin{bmatrix} +0.71 \\ -0.71 \end{bmatrix} \end{array} + \begin{array}{c} \textit{Stage} \\ \begin{array}{c} \text{I/II} \\ \text{III/IV} \end{array} \begin{bmatrix} +1.04 \\ -1.04 \end{bmatrix} \end{array} \tag{5}$$

For a female patient <50 years old, in Stage II, and presenting symptoms but classified as having MC, the estimated time would be 4.8 years (4.08 + 0.42 – 0.71 + 1.04 = 4.83).

Caution must be used in interpreting the individual coefficients in these equations. *All* factors must be included in the calculations to estimate median survival time. The equations cannot be used when the specified information is not known for all factors. The reason for this is that the coefficients of the factors are *not* independent in the statistical sense and therefore must be considered jointly. Had the analysis been based on one less factor, a different set of coefficients would have resulted for the factors considered.

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[5] For details concerning the estimation of parameters for this problem *see* Mantel and Myers (*4*).

The results of applying equations [4] and [5] for all possible combinations of these factors are shown in table 2. The values presented in this table are not reliable enough to be used for predicting survival for two reasons: 1) The number of cases included in each of the sex-stage-symptoms groups were not sufficiently large to measure the effect of each factor, and 2) the patients diagnosed during the later years (1967–68), who represent a large proportion of the NIH Clinical Center series, could only be followed into 1969. Thus we had only 1 or 2 years of observation on these patients.

TABLE 2.—*Estimated median years of survival for NS and MC classifications*

Histologic subtype and sex	Stage I/II with symptoms:		Stage III/IV with symptoms:	
	Absent	Present	Absent	Present
NS				
Male	8.9	4.6	7.7	3.4
Female	13.5	9.2	12.4	8.1
MC				
Male	5.4	4.0	3.3	1.9
Female	6.3	4.8	4.2	2.8

Although one must be cautious in interpreting these results, several interesting observations can be made. For both NS and MC patients, females had better survival than males. The advantage for females was much greater among the NS patients. Survival for NS patients is consistently higher than for MC patients, which suggests that the NS and MC classifications represent 2 different types of disease. This type of analysis raises issues regarding the survival advantage of female patients. For example, is the immune response stronger in females?

DISCUSSION

Two statistical methods for handling multivariate data have been presented using 270 Hodgkin's disease patients seen at the NIH Clinical Center. The first procedure, based on a *relative odds* technique, allows an evaluation of differences in survival for patients classified according to a specific factor while taking into account differences in their distribution with respect to 1 or 2 other prognostic factors. This procedure does not lend itself easily to an evaluation of survival if more than a two-factor adjustment is required. In addition, it requires that, for all intervals of observation, patients must be represented in all subcategories of classification for the factor(s) taken into account; otherwise little or no adjustment is achieved.

The multivariate analysis, based on the assumption of an exponential distribution, allows for joint evaluation of all factors thought to be associated with survival. It also requires that there be a sufficient number of patients in the various subcategories. Caution must be used in interpretation of the individual coefficients for estimation of the median survival time. All factors must be included in calculations based on equations [1] and [2] to estimate median survival time. The equations cannot be used when the specified subcategories are not known for *all* factors included in the analysis. Since the coefficients of the factors are not independent in the statistical sense, all factors must be considered jointly. Had the analysis been based on one less factor, a different set of coefficients would have resulted for each of the remaining factors.

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Criteria of Cure: Statistical Considerations [1,2]

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SUMMARY—Statistical models for fitting observed data on cancer survival have been proposed by a number of investigators. Three models are discussed which include parameters designating cured proportions of patient populations. Each model makes an assumption as to the shape of the survival curve for patients who are not cured, and each assumes that cured patients, if any, have survival experience similar to that of some appropriate comparison population free of cancer. The survival curves for the noncured fractions all approach 0 asymptotically, so there is no finite time at which all remaining survivors may be described as cured. Haybittle applied the above models to data for patients with Hodgkin's disease and demonstrated proportions cured significantly >0 for both localized and generalized disease. The above concepts of cure relate to survival experience only and in theory may include as cured some patients with persisting morbidity but without excess mortality. Concepts of cure are discussed in reference to freedom from evidence of the disease under treatment and freedom from ill effects of therapy, both of which may be investigated with the same models as have been used for survival. None of the models discussed distinguishes patients with a naturally benign course of disease from patients cured as an effect of therapy. —Natl Cancer Inst Monogr 36: 561–565, 1973.

LOCALIZED Hodgkin's disease is, to a very large extent, a malignant disease of young people in their 20's and 30's. The most important consideration to a patient with this disease at these ages is to know that he has some substantial likelihood of living a normal life after he completes a proposed treatment. This concept of living a normal life is fundamental to all the concepts of cure. There is, however, no general agreement as to how to recognize or quantitate the concept.

Statistical approaches to the concept of cure are generally concerned with determining how many patients are cured rather than with the question of which ones are cured, though both questions have been approached statistically.

One of the most common uses of the word "cure" is in the phrase "5-year cure." The concept here is that anyone who survives 5 years after diagnosis of a malignant disease is sufficiently unusual to be considered to be free of the disease. For most malignant diseases, it is clear that this is not a satisfactory measure. It does identify a number of particular individuals, but many of these are not, in fact, cured in the sense of having a normal life ahead of them. In general we simply have to reject the use of the word "cure" in connection with the 5-year survival.

In 1948 Boag (*1*) proposed a more sophisticated definition. Boag's method divided a group of cancer patients into hypothetical cured and noncured groups. It was supposed that the cured group had survival similar to that of a general population, while the noncured group had survival times fitting a log normal distribution. The survival curve of Boag had three parame-

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

[2] Supported in part by Public Health Service grant CA13607 from the National Cancer Institute.

ters, one of which was the proportion cured and the other two were the mean and standard deviation of the log normal distribution.

To determine the parameters, it is necessary to identify 4 clinical subgroups of the patient population. Surviving patients must be classified as to presence or absence of evident disease at the point in time chosen for evaluation. Patients who have died must similarly be classified as cases of death with cancer present and cases with cancer absent. Boag's formulation has had fairly limited use.

The model most widely used for quantitating cure has been that of Berkson and Gage (*2*). The basic concept of Boag was used by Berkson and Gage, i.e., that the cancer population could be considered to consist of cured and noncured fractions. Again the cured fraction was supposed to have general population survival. The noncured fraction was considered to have exponential survival. The Berkson-Gage formula has 3 important advantages over the Boag method. First, it involves only 2 parameters rather than 3. One parameter is the proportion cured, the same as with Boag's curve. The other is the constant of the exponential survival curve for the noncured fraction. A second advantage is that it is commonly possible to get good estimates of the 2 Berkson-Gage parameters, while it is often impossible to get converging estimates of the 3 Boag parameters. Third, and the point Berkson emphasizes most, is that it is not necessary to make any assumptions as to which deaths should be attributed to cancer and which surviving patients are free of evident disease. The Berkson-Gage formula usually gives a good fit to observed data throughout most of the survival curve. It does, however, often fit poorly the earliest part of the cancer survival curve, before the principal force of mortality from cancer begins.

Haybittle (*3*) proposed still another survival curve that involved an estimate of a cured proportion. Haybittle's formula has only 2 parameters, as with Berkson and Gage's. Conceptually, it differs in that it assumes that the force of mortality becomes steadily less year by year. This is in contrast to the exponential model of Berkson and Gage, with a constant force of mortality. For survival curves carried to very long follow-up periods, the Haybittle model generally fits the tail of the survival curve better than a Berkson-Gage curve does. In his 1959 paper, Haybittle proposed a solution that required identifying 3 clinical groups, i.e., survivors, deaths attributable to cancer, and deaths attributable to intercurrent disease. In later papers he adopted Berkson's position that only death and survival could be reliably identified. The proportion of deaths not attributed to cancer then came from survival data on the general population rather than from a differential classification of individual deaths as cancer and noncancer deaths.

All of these analytic methods were developed principally for study of breast cancer mortality, though the concepts were general. In 1963 Easson called attention to the fact that Hodgkin's disease was a malignancy that should be discussed within the context of cure (*4*). Easson and Russell proposed that, if the survivors after some period of time have the subsequent mortality experience of the general population, those survivors might be considered cured. Essentially the same concept was proposed in 1929 by Greenwood (*5*). As it was stated by Easson and Russell, this was similar in some respects to the 5-year cure rate. It identified, at least in theory, the actual individuals who were cured. It was superior to the 5-year cure rate in that there was no prior assumption as to when cure was to be achieved or as to whether it would be achieved in any particular malignancy.

The concept that Hodgkin's disease should be studied within the context of cure was extremely valuable. The particular formulation was unfortunate in that large populations had to be followed for long periods to demonstrate survival approximating that of the general population. More importantly, the test criterion for cure was a 0 slope, after some follow-up period, of the adjusted survival curve, defined as total survival adjusted for expected survival in a comparable general population. While a slope consistent with 0 might be observed in a given series of patients, an alternative interpretation consistent with the data would be a slope approaching 0 asymptotically over time. Easson and Russell's report was promptly challenged on essentially this basis, and it was noted that their data were consistent with a slope substantially <0. Indeed a later publication by Easson (*6*), expanding his original series, showed a slope significantly different from 0 for the interval 10–15 years after diagnosis.

Haybittle in 1963 (*7*) supported Easson's contention of cure but used the previously established methods of analysis. These methods envision a 0 slope only as a limit. The test criterion for cure is the proportion of the cancer population that has mortality experience similar to that of the general population. Using Easson's data, Haybittle showed that this fraction was significantly >0.

Table 1 shows Easson's data for localized Hodgkin's disease and Haybittle's estimates of percent cured, as derived from the Berkson-Gage model and from Haybittle's model. These 2 results, 36 and 32%, are in good agreement with one another, and both clearly exclude the value 0. Furthermore, Haybittle showed that his method of analysis did not force cancer data into a pattern which contradicted general clinical experience. For carcinoma of bronchus and for Stage 4 carcinoma of the breast, he showed a cure rate not significantly different from 0; i.e., some malignancies had cured fractions significantly >0 and others did not, and the particular sites and stages that were curable in this sense were those generally understood to be curable.

Surprisingly, generalized Hodgkin's disease was also found to be curable in Easson's data (table 2). The cure rate is only 13% with a standard error of 3. The true rate may be as low as 7% but is evidently not 0.

Survival data generally do not reveal the shapes of survival curves for quite a number of years of follow-up in a way that allows an estimate of cured fractions. Both the Berkson-Gage and Haybittle methods often give poor fits to the 1st year's survival. Both analyses assume maximal force of mortality at 0 time, and this is generally true in unfavorable tumors or un-

TABLE 1.—*Localized Hodgkin's disease: Percent survival, adjusted*

Period of treatment	Number of patients	Survival (yr)		
		5	10	15
1934–46	64	50.1	42.3	39.7
1934–51	137	56.0	45.2	—
1934–56	277	56.8	—	—

Percent cured: 36 ± 5 (Berkson-Gage)
32 ± 6 (Haybittle)

TABLE 2.—*Generalized Hodgkin's disease: Percent survival, adjusted*

Period of treatment	Number of patients	Survival (yr)		
		5	10	15
1934–46	127	16.2	13.5	11.6
1934–51	309	17.2	11.0	—
1934–56	545	17.8	—	—

Percent cured: 13 ± 3 (Berkson-Gage, Haybittle) favorable stages. In early clinical stages of most cancers, as with localized Hodgkin's disease, the experience of the 1st year is better, not worse, than that of later years.

Because of these difficulties with mortality analyses and because the concept of cure involves considerations of morbidity and of mortality, there has been an interest in morbidity analyses. Construction of lifetables with some morbidity event as endpoint is exactly analogous to mortality lifetables. Since morbidity events precede mortality, it may be hoped that the shapes of morbidity curves will become apparent at an earlier point in follow-up than do mortality curves.

The morbidity event most commonly used in discussions of cure is a first relapse. This measure has been particularly appropriate in studies of breast cancer following curative surgery, when all or almost all cases are free of evident disease at the end of the surgical procedure. A relapse refers to appearance of disease after a period in which it has seemed to be absent, a remission.

In evaluating cure following a modality of therapy with which evidence of disease disappears over a protracted time, some decision must be made as to the group of patients who will be considered eligible for a relapse. In Hodgkin's disease, most patients who have ever been observed to be free of evident disease reach this status by the end of a course of radiotherapy, usually over a period of 4–12 weeks. Some small additional groups will become free of evident disease over a number of further weeks, with or without further treatment. Musshoff and Boutis (*8*) evaluated patients at the end of treatment. Patients with persistent or recurrent disease at this date were considered not to have achieved remission, and thereafter they were considered not eligible for relapse, even though later remission may have been achieved. In a later paper (*9*), Musshoff et al. evaluated patients at 6 months after diagnosis. Patients free of evident disease at that date were eligible for subsequent relapse. Irrespective of the date of evaluation for remission, 0 time was taken as the date of diagnosis and patients not in remission at the evaluation date were treated as failures. This judgment was supported by the finding that the percent failures (initial failures plus subsequent relapses) at 14 years was essentially equal to the percent deaths. The implication was that failure to achieve early remission implied a very small probability of a 14-year survival.

Frei and Gehan (*10*) used relapse data reported by a number of authors and "have arrived at the position that patients who survive relapse free beyond 4 years have at least an 80% chance of cure." They did not describe the procedure for arriving at this estimate, but the most substantial series cited by these authors, and the only series showing observations beyond year 15 of follow-up, is that of Peters, for which the probability of remaining relapse-free from year 5 through year 20 was found to be 71.5%. In the follow-up interval of 16–20 years in Peters' series, there was a 2% relapse. Thus it is not clear that a 0 slope for the relapse-free curve is achieved within 20 years or that the relapse curve offers an earlier solution to the problem of cure than is possible with the survival curve.

I have used the first extension of disease beyond the initial sites of involvement as the morbidity event of interest (*11*). This has the advantage of being an event that can occur at any time after initial evaluation. In theory it would be undesirable to consider a patient with persistent disease in the initial site as cured, but in practice we have never seen a case of persistence for as much as 6 months without either remission or extension.

Early local extensions may respond completely to prompt courses of therapy, beyond the initial course, so that cure in the sense of continuous freedom from extension may be substantially less than cure in the sense of freedom from death or freedom from relapse, as discussed above. If distant extension, referring to extension across the diaphragm or to extranodal sites, is used as the morbidity event, a very satisfactory measure of cure may be found. No data are available at this time to indicate whether a useful estimate of cure can be obtained in a shorter follow-up using extension as the endpoint rather than death or relapse.

It will be clear that any measure of morbidity is more subject to observational error than is mortality. This is of particular concern if morbidity measures are used in comparative evaluation of several regimens of therapy. Rigorous attention must be given to the possibility that the therapy itself may influence the assessment of morbidity differently for different modalities of therapy.

In addition to mortality, relapse, and extension of disease, Frei and Gehan (*10*) noted that freedom from complications of therapy should also be considered within the concept of cure. This is clearly appropriate, and I am not aware of any analyses that have taken this factor into account. No new analytic problem is posed, though some attention would have to be given to problems of definition of complications and to uniform methods for their assessment.

One final concept of cure should be mentioned: cure in the sense of an effect of therapy in a cause-effect relationship. The analytic methods that have been described do not distinguish between a naturally benign course of disease and a case converted by therapy to a benign course. Berkson and Gage (*2*) cautioned about this distinction as follows: "Assuming the estimate (of cure) to be fairly accurate, it still may be argued that all or a portion of them would have lived as long as they did, even if they had not been treated." While spontaneous cures of many cancer sites have been reported, Hodgkin's disease has not commonly been found in such series. There have been reports of very long survival after what we now consider to be totally inadequate therapy. These, like spontaneous cures, are evidently rare, and probably most of the proportion cured, as determined by the various analytic methods discussed, are in fact cured by therapy. It is by clinical trials and related studies that the proportion cured by a particular treatment can be determined.

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Criteria of Cure: Clinical-Pathologic Correlations [1,2]

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SUMMARY—Of 10 patients who were symptom free for 10 years after initiation of therapy, 4 are alive without clinical evidence of Hodgkin's disease, 4 are dead without clinical evidence of Hodgkin's disease at the time of death, and 2 died with gross evidence of Hodgkin's disease when autopsied. In one of these, the disease was extensive and considered the underlying cause of death. Five of these ten patients were also relapse free for ≧10 years after initiation of therapy. Three of these are alive, and one is dead without clinical evidence of Hodgkin's disease. One patient died with extensive disease—documented at autopsy. It seems, therefore, that a symptom and/or relapse-free period of ≧10 years is not an absolute indication of clinical cure.—Natl Cancer Inst Monogr 36: 567–569, 1973.

THE DEFINITION of cure of Hodgkin's disease has been expressed in statistical (*1*) as well as in clinical terms (*2, 3*). According to the former, a patient population that is clinically free of the disease, and whose age-corrected death rate curve runs parallel to that of the general population, can be considered cured. According to the latter, relapse-free intervals of 5 or 10 years after initiation of radiation therapy indicate probable cure. If, however, one accepts the thesis that cure means complete eradication of the neoplastic process by whatever therapeutic modality, absolute proof that this has been accomplished in Hodgkin's disease is still lacking, though long relapse-free survivals strongly suggest it. Since we were unable to find reports of long survivals in which eradication of the disease was histolgically proved at autopsy, we undertook a retrospective study of long-term survivors among 280 patients with Hodgkin's disease admitted to the University of Chicago Hospitals and Clinics between 1931 and 1964. The results of this study have been previously reported (*4*) and are summarized in tables 1–4.

TABLE 1.—*Long-term survivors in Hodgkin's disease (4)*

Patient population	Number	Percent
Total No. of patients	280	—
Patients surviving ⩾10 years	40	14.2
Living	11	
Dead	29	

Although the patient population forming the basis for this study belongs to an era during which Hodgkin's disease was not adequately treated by present-day standards, 14.2% of the patients survived from 10 to 27 years. In 16 of 18

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TABLE 2.—*Autopsy findings in long-term survivors (4)*

Autopsy findings	Number
Evidence of Hodgkin's disease present	16
Evidence of Hodgkin's disease lacking	2
No autopsy performed	11

TABLE 3.—*Causes of death in long-term survivors (4)*

Cause of death	Number
Hodgkin's disease implicated as significant contributory cause	14 (12)
Indeterminable	8 (0)
Not directly attributable to Hodgkin's disease	7 (6)

*Numbers in parentheses indicate autopsy cases.

TABLE 4.—*Causes of death and lengths of survivals after diagnosis of Hodgkin's disease in 7 patients who died of diseases other than Hodgkin's disease (4)*

Cause of death	Survival (yr)
Generalized coccidioidomycosis*	10.3
Disseminated breast carcinoma†	11.3
Coronary occlusion	11.6
Pneumonia	12.3
Nephritis and hepatitis secondary to radiation	15.5
Malignant melanoma	20.6
Pulmonary fibrosis secondary to radiation*	27.0

*No evidence of Hodgkin's disease at autopsy.
†No autopsy performed.

patients autopsied, Hodgkin's disease was demonstrated histologically, and in the remaining 2, one cannot be certain that the tissue sections available for study were sufficiently representative to exclude with certainty the presence of microscopic disease. In 12 of the 16 patients in whom Hodgkin's disease was demonstrated grossly and/or microscopically, the disease was extensive at the time of death and considered to be an important primary or contributory cause of death. In the remaining 4 patients who died from other diseases (table 4), Hodgkin's disease was limited to one or several small microscopic foci and was not considered a contributory cause of death.

Thus it is important to emphasize that in the group of 16 long-term survivors in which Hodgkin's disease was discovered at autopsy, the extent and apparent activity of the disease appeared to be of 2 basic types: In 4 of the 16, only small foci of Hodgkin's disease were observed. These were limited in 2 patients to lymph nodes, in 1 to lymph nodes and spleen, and in 1 to lymph nodes and bone marrow, respectively. In these cases, the disease appeared to be of low-grade activity. In the remaining 12 patients, the disease was much more extensive and appeared to be of high-grade activity; death was directly related to Hodgkin's disease.

Because of the importance that has been attached to a 10 years' symptom- and/or relapse-free interval as strong clinical evidence for cure, we tried to correlate these features with the pathologic observations in our long-term survivors.

Of our patients, 10 had symptom-free intervals of at least 10 years. Of these, 4 are alive 21.7, 20.2, 17.0, and 13.7 years, respectively, after initiation of therapy. Of the 6 patients who are dead, 1 died with no evidence of Hodgkin's disease at necropsy (27-yr survival), 1 died from cardiac failure with Hodgkin's disease involving the thoracic cage and one adrenal (19.3-yr survival), and 1 died from septicemia secondary to chemotherapy. In this patient extensive Hodgkin's disease was evident at autopsy (11.9-yr survival). No autopsy was performed in the remaining 3 patients (15.5-, 11.5-, and 10.7-yr survival, respectively). The 1 with a 15.5-year survival died from rheumatic heart disease without clinical evidence of Hodgkin's disease and the causes of death of the other 2 are not known.

Five of these patients also had relapse-free intervals of >10 years. These included 3 of the 4 patients who are living (21.7, 17.0, and 13.7 yr) and 1 who died from rheumatic heart disease without clinical evidence of Hodgkin's disease at death, and on whom no autopsy was performed. In the fifth patient (11.9-yr survival), autopsy revealed extensive Hodgkin's disease.

Finally, the histologic subtype appears to be

TABLE 5.—*Histopathologic classification of Hodgkin's disease in initial biopsy specimen of 37 long-term survivors (4)**

Histopathologic classification	Number of cases	Percent of histologic type: In 37 long-term survivors*	In 255 of total group of 280 cases†
Lymphocytic predominance	5	13.5	12.6
Nodular sclerosis	28	75.7	37.8
Mixed cellularity	2	5.4	36.0
Lymphocytic depletion	0	0.0	9.5
Unclassifiable	2	5.4	4.1

*Three cases in which pretreatment biopsy specimens were not available were excluded.

†Twenty-five cases in which pretreatment biopsy specimens were not available were excluded.

significant in long-term survivors. Table 5 shows that the percentage of cases of nodular-sclerosing Hodgkin's disease in the long-term survivors was twice that in the original group, that in Hodgkin's disease with lymphocytic predominance it was about the same, and that in mixed-cellularity type it was less than one-sixth of that in the original series. No patient with lymphocytic depletion survived 10 years.

Of the 10 patients who were symptom free for at least 10 years after initiation of therapy, 7 had Hodgkin's disease of the nodular-sclerosis type, 2 showed lymphocytic predominance, and 1 mixed cellularity in the diagnostic biopsy. Of the 5 who were also relapse free during the same period, 4 had nodular-sclerosing Hodgkin's disease and in 1 the disease was of the mixed-cellularity type.

Our observations suggest that clinical cure of Hodgkin's disease does not necessarily indicate eradication of the disease but may represent a state of equilibrium between host and neoplasm, particularly in the patients with the prognostically favorable histologic types. This seems to be true in long-term survivors who die from unrelated causes, in whom small foci of Hodgkin's disease are discovered at autopsy. Extensive disease at autopsy in other long-term survivors suggests that this equilibrium may have been disrupted, eventually leading to death. Our reluctance to equate long survival with cure applies only to this and similar series in which therapy was usually palliative and tumoricidal doses of radiation were rarely, if ever, administered. Quite possibly, however, aggressive radiation therapy aimed at destruction of all tumor may completely eradicate Hodgkin's disease. Only the future will tell whether persistence of Hodgkin's disease in long-term survivors is a consistent biologic phenomenon or whether it occurs only in patients inadequately treated by present-day standards.

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Invited Discussion: Forcing a Sequential Experiment to be Balanced [1]

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TO ILLUSTRATE the need for a method such as I have devised (*1*), I presented a table of data that Henry Kaplan had brought to the Statistics Department at Stanford University 5 years ago. This experiment was not working out quite the way it was supposed to because the design was rather faulty. The table had two classifications: 1) age (10–19, 20–29, 30–39, and 40–49 yr) and 2) information as to whether the person was in the treatment or control group. In the youngest age group there were 7 treatments and only 2 controls; similar inequalities of distribution were evident in the other age groups.

Kaplan explained he had followed the advice of the Statistics Department and had completely randomized the experiment as it went along, as he was supposed to do. He had been given a set of sealed envelopes, each containing a slip of paper stating either "treatment" or "control." The assignments had been made essentially by flipping a coin. It may seem that Kaplan was the victim of bad luck in getting categories with such imbalances, but actually the probability in just that one age category of getting a 7:2 distribution or worse is 18%. Since there were 4 age categories, there was a very high probability in an experiment like this of getting a very bad imbalance between the number of treatments and controls in any one category. Analysis becomes quite difficult in a situation like this. One must rely too heavily on statistical techniques that theoretically balance things out.

Thus, it seemed of interest to consider, in a sequential experiment in which patients come in one at a time and are assigned immediately to either treatment or control, whether there is a way to make the number of treatments and controls in each category come out roughly the same without giving up the advantages of randomization. One method to avoid unbalanced situations is to block the experiment. For example, one might block in sizes of 10. Within each group of 10, 5 treatments and 5 controls are chosen randomly. Although this "permuted block" method is very useful, it has some disadvantages. It is quite unwieldy for an experiment with 4 or more different categories. Another disadvantage, a major one in a life-threatening situation like Hodgkin's disease, is that at certain points in the permuted block you know exactly what is going to happen next. For example, if the permuted block is of size 10 and you have already chosen at random 5 treatments and 2 controls, you know that the next person is going to be a control. This is just the kind of situation a statistician likes to avoid, because he then is in a difficult ethical bind and might be tempted to cheat. Cheating may take various subtle forms such as assigning a person to another category because he may be near a borderline in age, clinical stage, or some other criterion. Since it is known the person is a control if he remains in the category pertaining to that block, the statistician is tempted to say, "Maybe I was wrong about his classisification. After all, actually the patient is going to turn 20 in July—I'll put him in the other age category."

My method for dealing with this problem follows. At every stage one essentially flips a coin to see whether assignment is to treatment or control. However, one uses 2 different coins, one of which is not a fair coin. This "unfair" coin has a two-thirds probability of, say, being heads and a one-third probability of being tails. The first assignment in any category is made by a flip of the fair coin, so the treatment or control possibility is 50-50. If it comes up treatment, then the next case entered in that category is assigned to treatment or control with the other coin, with the probability of two-thirds control and one-third treatment. The coin can still come up treatment; now there are two treatments and no control in that category. In that case, the next flip of the coin again uses the biased coin with a two-thirds probability of control and one-third probability of treatment. Alternatively, one could have gotten a control assignment on that second flip, which is more likely after all, and then one would be back to the situation where there was the same number of treatments and controls and could use the fair coin again. Thus, this plan is really simple. Any time there is an imbalance, the biased coin is used, biased in the direction to correct the imbalance. When things are completely balanced, the fair coin is used. This plan, aside from being easy to implement, costs very little in terms of statistical reliability. That is, one is not likely to get into trouble because one is not completely randomizing—not using a fair coin after every point. At no point in the experiment is there ever a probability more than two-thirds of being able to say exactly what the next patient is going to get. This method is also superior to the method of permuted blocks in respect to several other criteria.

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SUMMARY OF INFORMAL DISCUSSION ON PROBLEMS OF CLINICAL TRIAL DESIGN AND IMPLEMENTATION

A. Laugier presented some of the experience gained in Continental Europe in the past 8 years in the design, implementation, and evaluation of a clinical trial in Hodgkin's disease. The story of this trial is told in a recent issue of the E.O.R.T.C.[1] Newsletter. As far as design is concerned, a clinical trial conducted by multiple cooperating centers is always a compromise. It is a compromise between the state of knowledge, the application of new techniques, the availability of diagnosis and treatment, and the personalities involved.

When they started out in 1964, lymphangiography was performed often, but laparotomy was not, and the new pathologic staging system was unknown in Europe until 1970–71. Initially, all Stage I and II cases were given the same options of treatment. Today, they would probably treat the unfavorable histologic types more aggressively. The kind of radiotherapy given in 1964 was also a compromise between the local radiotherapy performed by most of the radiotherapists at the time and extended-field radiotherapy, which many therapists were reluctant to use for Stage I and Stage II Hodgkin's disease. Finally, it was agreed to irradiate all the lymph-node areas located on one side of the diaphragm. There were problems with this because of the firm opposition of certain major centers. One center included only 10 patients in a 3-year period, then decided to submit all its patients to supplementary chemotherapy, and subsequently ceased to participate. Other centers took an active part in the protocol design and then withdrew from participation in the clinical trial. It was fortunate that enough centers in certain countries remained willing to participate long enough to complete the trial. They agreed to adhere to radiotherapy at a given dose level for a given period of time and not to change their mind for 8 years. However, they decided to give supplementary vinblastine for 24 months instead of 18 months when they realized how well tolerated it was. There was also the continuous temptation to use new drugs, even in the middle of a cooperative clinical trial.

As far as implementation is concerned, the question that arises is: "How many centers must participate—many or just a few?" On the one hand, one is tempted to increase the number of patients included and reduce the time required for the trial. On the other hand, only a few centers account for a substantial number of cases. In their experience just 3 centers out of 14 accounted for the bulk of the cases. **G. B. Hutchison** pointed out earlier the same experience, that most of the patients came from only 4 of 22 centers in the U.S. national trial. In fact, the choice between these 2 policies is not an easy one. It may be of interest from the political point of view to increase the number of participating centers to get more support, either medical or financial. Some isolated specialists can improve their local situation by joining a multicenter or a multinational cooperative clinical trial. Therefore, it is suggested that, insofar as possible, the door be kept open for any center that wants to join a trial.

As far as statistical evaluation of results is concerned, many unexpected and unplanned discrepancies and problems became apparent after several years. To begin with they had the feeling that management of the patient was not quite identical in each center, although the same protocol was ostensibly followed. There was room for subjective variation in several different areas. That is why 3 review committees were organized. The first committee was asked to review radiotherapy calibration procedures and to reduce discrepancies in dosimetric procedures used in the various centers. The second committee was composed of diagnostic radiologists who were experts in interpreting lymphangiograms. When a series of lymphangiograms were reviewed, 1 out of 5 initially thought of as normal by the local radiologist has been considered as questionable or positive by the review committee. The explanation for this lies in the fact that greater attention has been given to the upper para-aortic region after it was realized that most of the extensions they were seeing occurred in the upper lumbar para-aortic nodes. Also, some specialists were 7 years older and more experienced when they reviewed the lymphangiograms than they were when they initially interpreted them. The third committee was composed of 3 pathologists with special interest and expertise in interpreting malignant lymphomas. Their main concern was to achieve reasonably uniform interpretation of cases according to the Rye criteria. There was excellent agreement among the 3 pathologists for nodular sclerosis (94%) and mixed cellularity (95%), but less for the other two categories.

Excitement developed when they found, in a preliminary analysis, that better survival results were being obtained at Dutch centers at 1 and 2 years than at the other centers. However, a detailed analysis of the characteristics of the patients in the Dutch versus the other centers revealed a number of important differences. In fact, patients in the Dutch centers were younger than the others, systemic symptoms were seen less often, and they presented a greater proportion of cases of nodular sclerosis. These differences were statistically highly significant. Thus, considerable caution must be exercised when comparing results obtained in various places. In conclusion, the difficulties involved in design, implementation, and evaluation of clinical trials are no better understood than they were 10 years ago. However, these clinical trials have nonetheless greatly improved general standards of care. The management of the patient is less emotional and more scientific than it used to be. Among the many contributions of the Stanford University group to the understanding of diagnosis, staging, and treatment of Hodgkin's disease, perhaps the greatest lies in their introduction of randomized clinical trials in this field.

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[1] European Organization for Research on Treatment of Cancer.

Closing Summary: Goals for the Future [1]

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FIRST may I, on behalf of all those who have attended this Symposium, offer our thanks to the National Cancer Institute, the International Union Against Cancer, and the many institutes and hospitals that have allowed us all to travel and meet together. I am especially grateful to the Louise Buchanan Trust which sponsored two members of our unit. Special thanks are also due to Stanford University and to the Organizing Committee of Ronald Dorfman, Gregory O'Conor, and Saul Rosenberg—but most of all to our Chairman and Host, Henry Kaplan. Twenty-five years ago, on what was my second visit to the United States, I went with Professor Valentine Mayneord to see Egon Lorenz. He took us to meet a young man he thought we would find interesting and who was transplanting mouse thymuses. The Kaplan virus was being born. I was immediately impressed with the quality of Henry Kaplan's work and have enjoyed his company, worked with him, and benefited from his friendship, his example, and his advice ever since.

Today he has asked me to summarize this meeting and to indicate some goals for the future. All I can do is to pick out and comment on a few of the many points of interest. I shall not go through our deliberations in any detail, and I shall avoid referring to each of you by name, since I cannot possibly do justice to you all. This is the fifth meeting of this group, and I shall attempt to take a view of the position now gained.

DISCUSSION

To my mind this is the best type of meeting I attend, for here we arrange that a mixture of disciplines will be brought to bear on a single theme. I must admit to a twinge of regret at the loss of our old roundtable informality, but it is the penalty we pay for success. However, as we grow and accept change, we should do well to look critically at the pattern of discussion developing. At this meeting, for example, I have learned that "a discussant" is one who presents more data in less time. This I believe to be a pity. Thankfully, the days of authoritative statements declared without any foundation in fact are gone, but the days of data for the sake of data are not yet departed. It is well to remember that, when you talk to an audience composed of experts in many fields, the level of comprehension they may attain at a moment's notice in your field is not necessarily as brilliant as it would be in their own. The reasons for collecting the data presented, the hypothesis which is under attack, and the conclusions you think might be drawn should always be made clear. A discussant may then discuss, criticize conclusions, and relate the work that has been presented to the advancing level of understanding. Karl Popper's famous book, "The Logic of Scientific Discovery," and his later and equally remarkable work, "Conjectures and Refutations," should be required reading for all discussants. You must have noticed how the workshops and those few occasions on which we have taken a broad view of the implications of investigations put before us have noticeably raised the spirit of this meeting.

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[1] Presented at International Symposium on Hodgkin's Disease, Stanford University School of Medicine, Stanford, Calif., March 20–24, 1972.

We do not always draw the wisest conclusions from our own data, and one of the main functions of a friendly meeting such as this is to allow us to help each other make the most of what we have done and to plan our next steps more intelligently. The first time I came to the San Francisco Bay area was to see Ernest and John Lawrence in Berkeley. There I met Joe Hamilton, the only doctor ever to build a cyclotron. He was a great teacher. He ran a journal club for his students. At the meetings they were each given an article to report from which Joe had removed the author's conclusions; each student was expected to list his own conclusions and only when this had been done were the author's ideas about what the data might mean revealed. I adopted Joe Hamilton's method for a while and the differences in conclusions drawn from the same data by the authors and by my independent observers were most revealing—and not always in favor of the authors. Discussants at meetings such as ours have, I believe, a much more interesting and far more difficult task than most of them have attempted on this occasion.

First Goal for the Future: Discussants to be the most carefully selected and most competent members of the group, to have more time to speak, to present no new data unless these contradict or usefully expand what has gone before, and to be charged with setting about the task of advancing understanding.

TREATMENT

The treatment of Hodgkin's disease since this group first began its meetings has provided a steadily advancing success story. We are so pleased, so rightly pleased, with what has been achieved, that we must now beware of becoming too satisfied. It is most necessary that we consider the price paid for each advance and constantly look for what remains to be done. These points have indeed been honestly reviewed during this meeting, complications have been assessed, and weaknesses revealed. In a summary perhaps I can skip over the advances and the wide success which have been achieved by the raising of standards internationally, and confine myself to some remarks about two of the less obvious reasons for success:

1) The chief reason for success has not, I think, been due to linear accelerators, much though they have contributed, and outstanding in this type of radiotherapy though they are, nor to new drugs of special potency, for many of the old ones are still the mainstay of our chemotherapy, but to clinical oncologists—physicians, surgeons, and radiotherapists—working together with professional generosity, sharing their growing experience, testing their ideas by trials, and combining their various treatment methods to new advantage.
2) Another reason for progress, I believe, has been the growing acceptance of Hodgkin's disease as a system disorder with fairly predictable patterns of origin and spread which have led us to wider-field irradiation and to an increasing use of spaced, combination chemotherapy.

Before we become complacent, however, let us remember that we have not yet reconciled our views on the modes of spread of Hodgkin's disease—indeed, we have avoided all discussion of this controversial matter on this occasion—and that in treatment we have yet to learn when enough is enough.

Second Goal for the Future: So to solve the problems of modes of spread and of detection of small foci of disease, and so critically to survey the results of our trials, that we may better learn to treat each patient according to his need rather than by random numbers. This would avoid the giving of more and more at considerable cost to all and sundry what is only appropriate to the few.

EVALUATION

Diagnostic evaluation in Hodgkin's disease has improved by leaps and bounds. Lymphography was a huge step forward; scanning, if crude at present, has value; and laparotomy has changed the scene.

A minor point, perhaps, but I wonder whether the habit of calling this a "staging laparotomy" is altogether wise. The operation is a splenectomy with removal of samples of liver, bone marrow, and lymph nodes. It does indeed give us the pathologic stage of disease. But the operation is no trivial investigation, and it is therapeutic in two senses: Diseased spleens are removed and the findings affect treatment policy. Lymphangiography is a far better method than laparotomy for surveying the abdominal nodes at greatest risk, and such a survey is not limited to one point in time.

Despite real gains from sampling the nodes at less risk and the liver, it is the splenectomy which justifies routine laparotomy. There are risks, and there is a morbidity. A patient might face these hazards with more equanimity for the sake of a therapeutic rather than an evaluation procedure. Doctors who are not working in this field might more readily understand, and so accept the necessity for this operation, if presented with a more directly beneficial implication.

Do not forget that here at Stanford University School of Medicine, where they have taught us so much on this subject, 174 normal spleens have so far been removed from patients with Hodgkin's disease, and we have still to learn what the long-term consequences may be. In assessing low-risk groups where laparotomy may not be needed, the retrieval rate after recurrence must form part of the reckoning; it has hardly done so to date. The possibly unnecessary investigation, no less than the unnecessary irradiation or chemotherapy, should be kept under constant review if we are to get the best obtainable with the least excess.

Third Goal for the Future: A greater refinement in the assessment of individual risk and need, to be derived from our group studies and trials.

HISTOPATHOLOGY AND CYTOLOGY

These have been the foundation of our progress. Classification, tumor typing, and consultation leading to comparability of reporting have made us here present confident that we are talking about the same things. We owe much to the pathologists—who have also handed us our best guide to prognosis and shown convincingly that we are dealing with a progressive malignant process which may yet be arrested. We have seen that >90% of the nodular-sclerosis type of Hodgkin's disease remains in this category even while it slowly spreads and that occasionally it may lie quiescent until the patient has run his normal lifespan. Now we need to spread the word. Comparability of histologic reports—outside those leading centers where it has already been achieved—if extended round the world would greatly increase the value of epidemiologic studies. We need more oncology centers. They really have proved to be of immense value not only because cooperation makes them more efficient in treatment but also because they concentrate experience, allow trials, raise skills, and increase understanding in many aspects of disease.

We also need to know more about the varied cell populations in Hodgkin's disease. Happily, pathologists are increasingly concerned with dynamic studies, with function, and with derivation. Cells which "look the same but act different" are taking on a new meaning for us.

Fourth Goal for the Future: More consultation among competent histopathologists to achieve comparability of reports and a more precise characterization of cell populations in nodes, spleen, thymus, blood, and lymph in terms of behavior, derivation, chromosomes, reproductive capacity, and antigenicity.

EPIDEMIOLOGY

The first day of our Conference was perhaps remarkable, rather because we were discussing the biology of Hodgkin's disease at all than because of the conclusions drawn. Geographic pathology, if firmly based, should tell us much of importance about inherited susceptibility and environmental hazard. Poor conditions of life do seem to lead to Hodgkin's disease at an earlier age and with a greater proportion of the tumor types with worse prognosis. With better conditions, fewer children and more young adults are affected; at the same time evidence of host resistance appears, accounting perhaps for the second age peak in later life either through delayed progression or because an age-related increase in susceptibility develops in some of those who warded off an earlier threat. I regard the dual etiology explanation of the bimodal age curve of Hodgkin's disease as a most improbable one.

The variations in age and sex incidence so far shown across the world are not very great but may be sufficient to provide some clues to environmental factors. We saw from Israel some evidence for a variation in incidence between different ethnic groups living in the same environment. If we knew more about migrants, their selection by sex and age for migration, and the length of time they had been in the environment when epidemiologic data were collected, and if we were more sure of the reliability of histologic diagnoses in national surveys, much might be learned.

Clearly, susceptibility—whether inherited or acquired—must be of great importance in this relatively rare disorder. Varying susceptibility is present not only in individuals in a population but also in lymph-node groups within individuals.

Anecdotes about the occurrence of Hodgkin's disease in siblings, cousins, and inmates of certain houses have lately blossomed into a fascinating survey of case linkage by contact and by association. This now requires the most rigorous investigation, not only because of its own importance but also because of its emotive aspect. This study has the air of authenticity without the discipline of statistical analysis. If there are carriers, what is it that they carry? Epstein-Barr virus? Is it possible that susceptibility is more common than we had thought, but that it is the exciting factors which are rare? This seems unlikely. Some evidence exists for oral transmission in a low-susceptibility population. We need answers to these questions; no doubt we shall have them soon.

Fifth Goal for the Future: A more secure background in comparable histologic reporting which would give credance to the epidemiologic studies of Hodgkin's disease combined with an intensive investigation of case-to-case and of case-contact-case dissemination.

ETIOLOGY

Lastly, I must look at etiology; but before doing so, let me suggest some considerations on which speculation might be based.

1) Hodgkin's disease is a comparatively rare disturbulance of the cell-mediated portion of the immune system.
2) Its induction periods and its premalignant phase are ill defined, but the first is sometimes long and the second may be waiting there for recognition if we only look a little harder.
3) It has a relatively benign form, which, however, tends to progress in malignancy.
4) It tends to be arrested as nodular sclerosis, often in Stage II, particularly in young adults and especially in females.
5) When life is harsh, or perhaps when exposure to a particular environment is early, it is contracted sooner and its natural arrest is rare.
6) Impaired delayed-hypersensitivity reaction is found early in the disease and must have some etiologic significance.
7) There is evidence of an association with infectious mononucleosis.
8) There is the probability that dissemination occurs through close contact between susceptible individuals, some persons acting as carriers while having no evidence of the disease.
9) Tumor-associated antigens are found in high concentration in lymph nodes and spleen.
10) There is growing evidence that attacks are mounted between differing groups of immunologically competent cells.

Hodgkin's disease seems to me to be a progressive, tumorous disorganization involving the immune system. There may be a parallel between this situation and that of tumors of the endocrine glands and their target organs, where disturbances within the system may exert pressures leading to the development of tumors which in turn produce new endocrinologic abnormalities. In Hodgkin's disease we have disturbances of immunologically competent cells, which may arise from two directions. On the one hand, impaired host reaction may set the scene through primary immune deficiency, autoimmune disease, or therapeutic immune suppression. On the other hand, strange immunocompetent cells may arise from several causes, of which the EB virus—with its stimulus to lymphoproliferation—seems to be the most likely. In such a situation, a graft-versus-host reaction and a host-versus-graft response may lead to tumor formation—as Henry Kaplan and I suggested 13 years ago and as supported by the work presented to us at this Conference by Stanley Order (*1*). A Kaplan-Smithers cell with an Order antigen perhaps? Or would disorder antigen be better? The idea of immunocompetent cells at war, which we first suggested, is now further supported by electron microscopy. It can be seen in action, by use of phase-contrast microscopy and time-lapse cinematography, with the technique developed in the 1950's by Dr. R. J. V. Pulvertaft of the Westminster Hospital, London, and now redemonstrated by Dr. A. E. Stuart and Dr. J. G. McVie in Edinburgh. We have much evidence for the presence of abnormal lymphoid cells in Hodgkin's disease, for failing immune response, and for lymphocytic proliferation passing to lymphocytic depletion. We see fever, sweats, loss of weight, eosinophilia, amyloid in lymphoid follicles and in the malpighian bodies of the spleen, and many other things which seem to fit the Kaplan-Smithers hypothesis.

As soon as this hypothesis was proposed, we set out to test it by giving parental spleen cells to F_1 hybrid rats in 1959, and in 1960 we had our first lymphoid tumor with leukemia after the animal had become refractory to further challenge. We have also shown in my laboratory that the factor which protects the F_1 hybrid once challenged by parental strain lymphocytes from further challenge by lymphocytes from either parental strain can be transferred passively to a previously unchallenged F_1 hybrid. Here perhaps we may have an autoantibody directed against the host's own lymphoid system.

My Sixth and Last Goal for the Future: This is the comprehensive one of encouraging attacks on the Kaplan-Smithers hypothesis by experimental means, perhaps by purifying the Order antigens, preparing pure antisera, identifying the cells carrying the antigen, devising a radioimmunoassay, and so providing us all with firm etiologic explanations and reliable diagnostic tests.

There seems to be plenty to be done before we meet again.

Once more, on behalf of you all, let me thank Henry Kaplan and his committee for a wonderful meeting.

REFERENCE

(*1*) ORDER SE, CHISM SE, HELLMAN S: Hodgkin's disease-associated antigens: Studies on segregation and specificities. Natl Cancer Inst Monogr 36:139–145, 1973

CLOSING REMARKS

I am sure that all of you join me in thanking Sir David for a splendid summary. It remains only for me to express my thanks to many, many people for the success of this symposium. I would like again to acknowledge the support of the International Union Against Cancer, the National Cancer Institute, and the various individual institutions that have helped by sending some of their people here. I would also like to acknowledge the help of my colleagues on the Organizing Committee, especially G. T. O'Conor, who really did a great deal of work on the first day's program, as well as Ronald Dorfman and Saul Rosenberg. Our Conference Secretaries, and in particular my secretary, Miss Ruth McConnell, and Saul Rosenberg's secretary, Miss Vreni Ackerman, have given invaluable assistance in the organization of the many details of the meeting and our departmental Administrator, Mr. Richard Placone, has attended to many of the more mundale details that help make any such meeting a success. We also owe anticipatory thanks to the transcribers who have not yet started their work, but who, I hope, will do an outstanding job in providing an intelligible typescript of all that has been said this week. I would also like to express our thanks to our valiant projectionist back there in the booth. I think you will all agree that, with so many papers and invited discussions, he has really done a remarkable job of keeping the slides in order. Finally, I would like to express my personal appreciation to all the speakers and the discussants for their outstanding presentations and for their cooperation either already expressed or soon to be expressed in submitting their finished manuscripts. The meeting is adjourned.

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